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## A BIOCHEMICAL STUDY OF RESISTANCE TO MILDEW IN OENOTHERA [1]


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## INTRODUCTION

### THE NEED OF BIOCHEMICAL DATA IN THE SOLUTION OF THE PROBLEM OF IMMUNITY AND SUSCEPTIBILITY TO DISEASE IN PLANTS

The researches of recent years on the physiology of parasitism prove the ever-increasing need of biochemical data in the solution of the complex causes of immunity to disease in plants [see Appel(2) and Butler(8)]. Thus, Ward,(50) after having reviewed the literature dealing with the parasitism of fungi from the time that De Barry, Pringsheim, Koch, and Pasteur established the foundation of the parasite or germ theory of disease, reviews his own work on the Uredineae and makes the following statement regarding the conditions of parasitism in this group:

> Infection, and resistance to infection, depend on the power of the fungus protoplasm to overcome the resistance of the cells of the host by means of enzymes or toxins; and reciprocally, on that of the protoplasm of the cells of the host to form antibodies which destroy such enzymes or toxins, or to excrete chemotactic substances which repel or attract the fungus protoplasm.

Massee(31) holds the opinion that—

> the entrance of the germ tubes of a parasitic fungus into the tissues of a living, healthy plant depends on the presence of some substances, in the cells of the host, attractive to the fungus. In other words, infection is due to positive chemotaxis.

Wagner(49) indorses the view of Ward, saying that in some healthy plants three classes of antibacterial products are formed; namely, agglutinin, lysin, and substances limiting the multiplication of spores and bacteria.

Cook(12) reports that the loss of resistance to the attack of fungi shown by certain fruits after they have been removed from the trees is correlated with a decrease of enzymatic activity. Further, in collaboration with Wilson(14) he observes the toxic action of tannins and vegetable acids on the germinating spores of *Endothia parasitica* and related species.

Mains(30) indicates the close relationship of rust infection to the carbon metabolism of the host plant when he observes that, in the absence of light, the rust *Puccinia sorghi* is not able to develop in corn seedlings or pieces of corn leaf which have been deprived of their soluble carbohydrates. He, therefore, suggests that the obligate parasitism of the rusts is perhaps due to their requirement of particular organic compounds contained in the living host.

Gustafson (19) finds that the respiration of *Penicillium chrysogenum* increases up to a certain point with the increase of the hydrogen ion concentration of the medium and that the hydroxyl ion greatly inhibits respiration.

Hawkins and Harvey (21) observe that *Pythium debaryanum* Hesse secretes an enzyme that dissolves the middle lamella of the host tissues, and a toxin that kills the cells of the potato tuber; but nonchemical factors are also concerned with infection, for by a mechanical device these experimenters were able to prove that more pressure is required to puncture the tissues of the fairly resistant strains of the tuber than is required for the susceptible ones. More recently, Brown (7) reports that certain volatile substances given off from plant tissues have a distinct effect on the germination of the spores of *Botrytis cinerea*, some having a stimulating and others a retarding effect. He also obtained similar results by using ethyl acetate and various essential oils.

In another direction, where the specificity of plant pathogens in relation to their hosts is involved, we find Reed (40) pointing out the physiological specialization of parasitic fungi, and Thomas (48) presenting data to show that infection of *Apium graveolens* by *Septoria apii* is favored by conditions which accelerate the growth of the host. Raines, (39) in experimental work on certain rust diseases of the higher plants, concludes that the vegetative vigor of the host is a factor which influences its susceptibility and resistance to rusts. Allen, (1) as a result of her recent cytological studies of the infection of resistant and susceptible wheats by *Puccinia graminis tritici*, confirms the hypothesis that "immunity is due to definite antagonistic chemical interactions between host and parasite."

These various experimental findings are of extreme importance in the biochemical consideration of disease resistance. They prove two important points; first, the existence of particular chemical substances in plants which may be either toxic or stimulative to parasitic fungi and, second, the dependence of parasitic fungi upon specific biochemical conditions within their hosts. From a biochemical standpoint it is not hard to understand how certain highly specialized fungi are restricted to particular hosts, because only those hosts provide the special compounds used in their nutrition, or perhaps because only certain ones among a narrow range of possible hosts fail to secrete inhibiting substances when infection takes place.

Some very significant results have already been obtained in the biochemical study of immunity in plants, as may be seen from the extensive literature reviewed by Willaman and Sandstrom(51) coincidently with the publication of their own investigations on the biochemistry of plant diseases.

Jones(25) found cabbage to be resistant or nonresistant to the attacks of *Fusarium conglutinans;* Peltier(37) found wild relatives of the cultivated citrus fruits immune to citrus canker; Christensen(9) demonstrated the resistance of certain varieties of swede to *Plasmodiophora brassicae;* and Norton(34) developed strains of asparagus resistant to *Puccinia asparagi.*

Turning to the powdery mildews, Salmon (42, 43) found striking contrasts in the resistance of various species of *Hordeum* and of the genus *Bromus.* Klaphaak and Bartlett(27) found the same situation in strains and hybrids of *Oenothera* which are often the hosts of mildew strains referred to *Erysiphe polygoni* DC. It was the material of crosses prepared especially for the last-mentioned investigation that was made available to me.

## THE OBJECT OF THE INVESTIGATION

This investigation concerns the problem of resistance to mildew in the genus *Oenothera.* The chemical compositions of several resistant and susceptible strains were compared, in order to discover whether or not there were definite correlation between composition and reaction to mildew. It was recognized, of course, that immunity might have a true biochemical basis, even if the gross analytical data failed to show differences. The power of a plant to secrete antibodies would not, for example, be indicated by such analytical work as that undertaken in the present investigation. If, on the contrary, there were large differences in the concentration of soluble carbohydrates or nitrogenous constituents, of such a nature as to have some obvious relationship to the nutrition of the fungus, the results would be of value. Again, differences in acidity, in the concentration of tannin, or of inorganic constituents, might be interpreted, if found, as a basis for corresponding differences in resistance. It was suspected, of course, when this investigation was undertaken, that such differences in gross chemical composition would be found. Whether they were or not, however, the analytical data would still be valuable for the light they would throw on the inheritance of chemical characteristics, on the fluctuating variation in chemical composition of the

same strain from year to year, and on the correlation between chemical composition and morphological characteristics of the several strains. The material was chosen so that the results would throw light on some of these matters, even if the chemical basis for disease resistance should not be disclosed.

### THE ADAPTABILITY OF OENOTHERA FOR BIOCHEMICAL STUDIES OF DISEASE RESISTANCE

The choice of *Oenothera* for the investigation was determined not only by the sharp distinctions in disease resistance shown by the various strains, but also by the fact that there was available a great deal of genetically known material. Furthermore, the results would fit into a large body of genetical data regarding the same material and the usefulness of the results would thereby be enhanced. It was by no means necessary to use *Oenothera*, however, for strains differing with regard to disease resistance are found in most groups of plants.

It was possible to secure samples of resistant and susceptible *Oenothera* strains grown under identical conditions and collected at the same time, and thus to reduce to a minimum the possible effects of external factors. Klaphaak and Bartlett(27) noticed that—

> nothing is more characteristic of the various elementary species and hybrids than the great differences that they show in susceptibility to infection by mildew. * * * the differences shown by certain pairs of reciprocal hybrids, were astonishingly definite, the one being white with mildew and the other absolutely free, although both were grown in adjoining rows under identical conditions, and often with interlocking branches.

Moreover, in *Oenothera*, resistant strains or susceptible ones may be obtained at will, since resistance is inherited as a unit factor, and the rules governing the inheritance of the factor have been worked out for several strains in accordance with the hypothesis of heterogametism put forward by Bartlett(5) and by Cobb and Bartlett.(10) The experimental findings with regard to mildew resistance conform exactly to theoretical expectations and justify the zygotic formulae assigned to the strains by Klaphaak and Bartlett. It is not deemed necessary to explain here how immunity and susceptibility in *Oenothera* are formulated on the basis of this hypothesis, since such information has been published in the papers referred to. For the same reason I have omitted the descriptions of the morphological external characters of the elementary species and hybrids that I used for my experiments, as well as discussions of the

mildew which infects them, since such data are either on record already, or soon will be through the publications of co-workers.

It may be said, however, that in the strains which I have grown for my studies I noted that, in some cases of reciprocal hybrids, the mildewed one is vegetatively more vigorous than the resistant one. On the other hand, the leaves of the resistant hybrid are darker green and tougher than the susceptible one. It might seem offhand that the differences in susceptibility and immunity could be accounted for by some morphological characters of the forms, but Klaphaak and Bartlett,(27) after examination of sections of leaves from the five different species and hybrids chosen, stated that no such morphological differences of the leaves could be detected. Salmon,(42) in his studies of the barley mildew, *Erysiphe graminis* DC., presumably did not find any morphological differences of the host plant that would explain resistance, since he stated that "susceptibility and immunity were due to constitutional (physiological) peculiarities and not to any structural ones." De Istvanffi and Palinkas,(16) in their pathological studies of the vine mildew, *Plasmopara viticola*, express the opinion that—

> the susceptibility of the plant depends upon the supply of water in the organ, the vapour tension in the stomatic chambers and intercellular cavities generally, the turgescence of the cells, and to some extent on the chemical composition of the cell sap.

In view of these facts, the conclusion seems warranted that the resistance to infection by powdery mildew shown by a number of plants must have a physiological or biochemical explanation.

## SOME PREVIOUS RESEARCHES BEARING ON THE PROBLEM

There has been no previous work, so far as I am aware, that deals with the biochemistry of resistance and susceptibility to any form of powdery mildew. Three papers which concern various aspects of the mildew problem are of interest in this connection. These are by Spinks,(46) Montemartini,(33) and Laurent.(28)

Spinks studied the effects of some inorganic salts on susceptibility of barley to mildew (*Erysiphe graminis*), and of wheat to yellow rust (*Puccinia glumarum*). He grew resistant and susceptible varieties of these two plants in Detmer's nutrient solution, with varying amounts of different salts. He concluded (*a*) that when the plants were provided with a large amount of nitrogen either in the form of ammonium salts or

nitrate, their susceptibility was increased, (*b*) that with lithium salts and mineral manures, especially the ones rich in potash, susceptibility was reduced, and (*c*) that plants which were semistarved with respect to nitrogen exhibited a considerable degree of immunity. Since Spinks's object was not strictly biochemical, he did not determine the amount of the different salts absorbed by the plants, or what became of the salts after they were absorbed. His findings, however, indicate the relationship of the nitrogen nutrition of the host plant to its susceptibility to the disease.

The relation of the nitrogenous constituents of the plant to mildew infection was also noted by Montemartini.(33) He observed that the water-soluble nitrogen in the leaves of the varieties of American oak resistant to powdery mildew constitutes only a little over 0.1 of the total nitrogen in the leaves. He said that this confirmed the previously published report of Pantanelli(35) that, in the susceptible oaks, the soluble nitrogen represents from 0.4 to 0.7 of the total nitrogen, while in resistant oaks it does not exceed 0.3.

Laurent(28) reported that severe infection of grapevines was noticed in the vineyards which had been manured directly with farmyard manure in the same year that the infection occurred, whereas in the ones in which phosphate and potassium fertilizers were used, infection was considerably reduced. Resistance was also found to vary according to the method of pruning and nipping, and the nature of the stock. He considered, therefore, that "all these factors act indirectly by modifying the chemical and physical qualities of the vine cells, resistance being due to the increase of the concentration of the vine-sap."

The strains of *Oenothera* used in the investigation are listed in Table 1.

## MATERIAL

TABLE 1.—*List of elementary species and hybrids studied.*

| Key No. | Culture No. | Name of strain. | Characteristic of strain. |
|---|---|---|---|
| 1 | 0847 | | |
| 1a | 1827 | Resistant *Oe. pratincola* (a segregate from species hybrid) | Resistant. |
| 1b | 2960 | | |
| 2 | 0822 | | |
| 2a | 1824 | Mildewed *Oe. pratincola* (a segregate from species hybrid) | Susceptible. |
| 2b | 2959 | | |
| 3 | 0864 | | |
| 3a | 1826 | *Oe. pratincola* hyb. *immunis* | Resistant. |
| 3b | 2956 | | |

TABLE 1.—*List of elementary species and hybrids studied*—Continued.

| Key No. | Culture No. | Name of strain. | Characteristic of Strain. |
|---|---|---|---|
| 4 | 0823 | | |
| 4a | 1825 | Mildewed *Oe. pratincola* (a segregate from species hybrid) | Susceptible. |
| 4b | 2954 | | |
| 5 | 0830 | | |
| 5a | 1804 | *Oe. mississippiensis* × *Oe. pratincola* hyb. *immunis* | Resistant. |
| 6 | 0820 | | |
| 6a | 1805 | *Oe. pratincola* hyb. *immunis* × *Oe. mississippiensis* | Susceptible. |
| 7 | 0829 | *Oe. pratincola* hyb. *immunis* × *Oe. mississippiensis* | Do. |
| 8 | 1818 | | |
| 8a | 2966 | *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis* | Resistant. |
| 9 | 1819 | | |
| 9a | 2963 | *Oe. pratincola* hyb. *immunis* × *Oe. cinerescens* | Susceptible. |
| 10 | 0838 | *Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *immunis* | Resistant. |
| 11 | 0821 | *Oe. pratincola* hyb. *immunis* × *Oe. "biennis Chicago"* | Susceptible. |
| 12 | 0814 | *Oe. cinerescens* × *Oe. mississippiensis* | Resistant. |
| 13 | 0827 | | |
| 13a | 1800 | *Oe. mississippiensis* × *Oe. cinerescens* | Susceptible. |
| 13b | 2957 | | |
| 14 | 1802 | | |
| 14a | 2958 | *Oe. cinerescens* × *Oe. mississippiensis* (metaclinic hybrid) | Do. |
| 15 | 0299 | *Oe. pratincola* × *Oe. reynoldsii*<br>(*Oe. pratincola* hyb. *amycosa*) | Resistant. |
| 16 | 0177 | *Oe. reynoldsii* × *Oe. pratincola* | Susceptible. |
| 17 | 0842 | | |
| 17a | 1816 | *Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx* | Do. |
| 17b | 2962 | | |
| 18 | 0849 | | |
| 18a | 1817 | *Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago"* | Do. |
| 18b | 2961 | | |
| 19 | 0831 | | |
| 19a | 1806 | *Oe. mississippiensis* × *Oe. "biennis Chicago"* | Do. |
| 20 | 0841 | *Oe. "biennis Chicago"* × *Oe. mississippiensis* | Do. |
| 21 | 0839 | | |
| 21a | 1807 | *Oe. "biennis Chicago"* × *Oe. mississippiensis* | Do. |
| 22 | 0833 | | |
| 22a | 1810 | *Oe. mississippiensis* × *Oe. pratincola* hyb. *rubricalyx* | Do. |
| 23 | 0848 | | |
| 23a | 1811 | *Oe. pratincola* hyb. *rubricalyx* × *Oe. mississippiensis* | Do. |
| 24 | 1346 | *Oe. pratincola* mut. *nitidissima* | Resistant. |
| 24a | 1348 | *Oe. pratincola* mut. *nitidissima* | Do. |
| 25 | 1347 | *Oe. pratincola* mut. *nitidissima* | Susceptible. |
| 25a | 1349 | *Oe. pratincola* mut. *nitidissima* | Do. |
| 26 | 199 | *Oe. numismatica* | Resistant. |
| 27 | 1191 | *Oe. pratincola* mut. *simulans* | Do. |
| 28 | 1192 | *Oe. pratincola* mut. *simulans rubricalyx* | Susceptible. |
| 29 | 1829 | | |
| 29a | 2953 | *Oe. pratincola* hyb. *immunis* | Resistant. |
| 30 | 1231 | *Oe. reynoldsii* × *Oe. pratincola* | Do. |
| 31 | 167 | *Oe. pratincola* | Susceptible. |
| 32 | 1828 | *Oe. mississippiensis* | Do. |
| 33 | 1234 | *Oe. reynoldsii* | Resistant. |
| 34 | 2952 | *Oe. pratincola* hyb. *rubricalyx* | Susceptible. |
| 35 | 2950 | *Oe. "biennis Chicago"* | Do. |

COLLECTION OF MATERIAL

In all my experiments the leaves only were analyzed, because they are the parts of the plant which are most severely attacked by the mildew, and are mildewed earlier in the season than the stems. There seemed no object in carrying all the work through in parallel with stems, since the large amount of woody tissue would render variation in soluble constituents less easily detectable.

The first year's collection was made during the early part of September, 1920. Some of the leaves of the cultures had already shown signs of chlorophyll degradation, but the mildew could still be seen on the leaves of the susceptible strains. Since this was the time when the problem was planned, I decided to get the materials that were available and use them for finding out whether or not chemical differences between resistant and nonresistant leaves persisted. The data were, of course, only to be used as amplified by material of the following year, collected earlier in the season.

Individual plants of all strains selected during the first year were self-pollinated, and their progenies were grown in 1921. Leaves were again collected, but this time they were picked during the middle part of August, the period when the plants were observed to be in the developmental stage and when the mildew was most active. In this year I also collected leaves from some strains not represented in my first year's collection, for purposes that will be explained later on. In the third year, 1922, the cultures were confined to five pairs of reciprocal hybrids which were self-pollinated in 1921, and to three elementary species. The five pairs of reciprocal hybrids were the ones that had provided the best material during the first two years, while the three elementary species were genetically the same as the parents of the three pairs of reciprocal hybrids. The first year's analyses were made of hybrids already in the garden when the work was planned. The parents had been grown the year before. For complete data it was necessary to analyze material believed to be morphologically and genetically identical with the actual parents of the hybrids. Such strains had been carried on in the garden cultures, and provided material for analysis in the third year of the experiment after it had been conclusively shown that year after year the various strains maintain their chemical characteristics with the same fidelity that they maintain their morphological ones.

Two separate collections of leaves were made this time (1922), the first one from July 5 to July 19 when the susceptible strains were not yet infected by the mildew, and the second one from August 23 to August 31 when the susceptible strains were heavily infected.

The leaves were picked one by one from the main stems and branches of the plants and care was taken that a fairly representative portion of the full-grown leaves of each plant was collected, and that those plants in the cultures which appeared to be phenotypically different from the rest (twins or mutations) were left untouched. The number of individual plants in a culture representing one strain varied from one hundred to three hundred, and the leaves collected from all of them were mixed and rapidly dried in the sun. The dried leaves were placed in clean cardboard boxes and stored temporarily until they could be ground. They were milled to pass an 80-mesh sieve. The fine powder was then kept in dry glass jars. To make the material as comparable as possible, reciprocal hybrids were collected the same day, between 9 o'clock in the morning and 6 o'clock in the evening. It was not found necessary to brush the leaves for the purpose of removing any sand that might adhere to their surfaces for, since they were picked one by one, it was possible to avoid taking those which were not free from particles of foreign matter.

## PLAN OF THE INVESTIGATION

The strains used can for convenience be considered in five groups (see Table 1). The first group contains the twelve strains and elementary species that were the parents of the reciprocal hybrids. The second group consists of seven pairs of reciprocal hybrids in which one hybrid of each reciprocal pair is resistant and the other one is susceptible. The third group is made up of three pairs in which both reciprocal hybrids are susceptible. The fourth consists of four strains of a single mutation, two of the four susceptible and the other two resistant. The fifth is composed of pairs of strains that have the same genetic composition but different pedigrees. These different groups of strains were used in order to answer the following questions: (*a*) Is there any difference in chemical composition between the resistant and susceptible strains of *Oenothera?* (*b*) If so, is there the same contrast in the chemical composition between reciprocal hybrids unlike in resistance to mildew that there is between wild elementary species of unlike resistance? (*c*)

In cases of mutations arising from the same parents and differing from one another with regard to resistance and susceptibility, are the visible differences between the forms paralleled in the chemical characteristics? (*d*) How does the chemical composition of their parents compare with that of hybrid progenies? (*e*) If two strains are judged by morphology and breeding behavior to have the same genetic composition, will they have also the same chemical composition?

Biochemical data bearing upon these five points were all desirable before any deduction could be made as to whether or not a chemical basis for resistance and susceptibility could be detected by the usual procedure of chemical analysis. It was planned to confine the investigation to the quantitative determination of the well-known chemical constituents of the plants, leaving for future consideration the study of specific constituents, if such there seemed to be. In order to reduce the analytical work, only four pairs of reciprocal hybrids were used in the study of the nitrogen distribution, the determination of carbohydrates, and the ash analysis.

Before proceeding to the description of the analytical methods adopted, I should explain why the study of the dried leaves was not supplemented by parallel investigations of the juices from the fresh plants. The juices were found to be so mucilaginous that the mechanical difficulties of obtaining sufficient material would have been almost insuperable in the case of an extensive investigation covering a large variety of strains. It seemed more logical to examine the tissues as a whole, leaving to the future more detailed researches, if the preliminary survey should indicate promising points of attack. More refined methods could be applied to more restricted material—perhaps a single contrasting pair of mutations or reciprocal hybrids, or a parent species and one of its mutations.

I am aware that during the process of drying there might have occurred autolytic changes which alter the chemical composition of the leaves. Since, however, the leaves were collected during summer days, they could be rapidly dried in one day so as to reduce autolysis to a minimum. Furthermore, since the experimental results were intended for comparative study any modification of the constituents of the leaves by autolysis would presumably be more or less of the same magnitude in all strains, because all the leaves were dried in the same way. Parkin(36) has studied autolytic changes in the composition of leaves during drying in connection with his studies of the car-

bohydrates of the foliage leaf of the snowdrop. He carried out two sets of experiments. Two equal portions of fresh leaves were taken. One portion was dried in the ordinary way, and the sugars from the dried leaves were determined. The other portion was immersed at once in liquid air, then withdrawn, and ground up while frozen and brittle. The powder was at once thrown into hot water to kill the enzymes, a little ammonia added to neutralize any acidity, and the sugars were determined in the aqueous extract. This procedure was designed to eliminate autolysis. The treatment with liquid air was merely to facilitate powdering of the leaves. It was the heat treatment, of course, which destroyed the enzymes. He found that the proportion of sugars in both cases is almost the same, as can be seen from Table 2.

TABLE 2.—*Parkin's data for different methods of sampling leaves for sugar analysis.*[a]

| | Sucrose. | Hexoses (reducing sugars). | Total sugar. | Ratio of sucrose to hexose. |
|---|---|---|---|---|
| Experiment I: | | | | |
| Fresh leaf | 12.84 | 5.94 | 18.78 | 1:0.46 |
| Dried leaf | 12.74 | 5.67 | 18.41 | 1:0.44 |
| Experiment II: | | | | |
| Fresh leaf | 10.46 | 12.87 | 23.33 | 1:1.23 |
| Dried leaf | 10.42 | 12.38 | 22.80 | 1:1.19 |

[a] The amounts of the sugars are calculated for 100 grams of dry leaf.

Any autolysis during drying would almost certainly have been reflected in the sugar ratios, since these afford an exceedingly sensitive indication of chemical changes going on in the cell. Parkin's work appears to justify fully the procedure adopted in sampling.

## METHODS OF ANALYSIS

*Moisture.*—Duplicate samples of the powdered leaves, the weight varying from 1.5 to 2.0 grams, were heated in a vacuum oven at from 90° to 95° C. for six hours. The loss of weight was taken as the moisture content.

### THE NITROGEN PARTITION

*Total nitrogen.*—It was decided to use a combination of the Gunning method modified to include the nitrogen of nitrates (26) and the iodometric method of Willard and Cake (53) for the determination of amino nitrogen in organic substances. The

nitrate nitrogen of the sample was retained by using salicylic acid and the nitro compound resulting from this treatment was reduced with sodium thiosulphate. The digestion was then carried out in the usual way with concentrated sulphuric acid and potassium sulphate, except that after the addition of potassium sulphate boiling was continued only for an hour at most. The digestion was completed by the addition of potassium persulphate free from ammonium salts, the amount varying from five to ten times the weight of the sample taken. When the destruction of the non-nitrogenous organic matter was complete, the acid solution was made alkaline with sodium hydroxide and the ammonia determined by oxidation to nitrogen with standard sodium hypobromite solution. This was done by adding an excess of the hypobromite to the alkaline solution and titrating the excess back with 0.2 *N* sodium thiosulphate after the addition of potassium iodide and hydrochloric acid.

*Ammonia nitrogen.*—The determination was made according to Grafe's method.(18) Ten grams of the sample were transferred to a long-necked 500-cubic-centimeter Kjeldahl flask, together with 20 cubic centimeters of concentrated sodium chloride solution, 10 cubic centimeters of 95 per cent ethyl alcohol, 100 cubic centimeters of distilled water, 10 cubic centimeters of saturated sodium carbonate solution (or enough to render the mixture alkaline), and 0.3 cubic centimeter of caprylic alcohol. The mixture was subjected to distillation in vacuo at a temperature ranging from 35 to 37° C. for three hours. The distillate was caught in a known volume of 0.1 *N* sulphuric acid, and the excess acid was titrated back with 0.1 *N* sodium hydroxide, using methyl orange test solution as an indicator. Caprylic alcohol was used to prevent excessive foaming, since it was found that the amount of ethyl alcohol indicated by Grafe was not sufficient for the purpose. A blank determination was carried out and the result subtracted from that given by the sample.

*Protein nitrogen.*—The protein nitrogen was determined according to Stutzer's method as adopted by the Association of Official Agricultural Chemists for albuminoid nitrogen.(26)

*Free amino acid nitrogen.*—Two or three grams of the powder were weighed, placed in a 300-cubic-centimeter Erlenmeyer flask, 100 cubic centimeters of distilled water added, and the flask shaken in the shaking machine for half an hour. The content of the flask was transferred to a Buchner funnel and filtered. Of the aqueous extract, two aliquot portions of 10 cubic centi-

meters were pipetted and used for the determination according to Van Slyke's method.(56)

The rest of the nitrogen, namely, the nitrate, acid amide, humin, basic, nonbasic, and peptide, was determined in the aqueous extract which was prepared from the leaves in the following way:

A 15-gram sample of the powdered leaves was placed in a liter beaker and boiled for thirty minutes with 500 cubic centimeters of distilled water. When cold, the clear supernatant liquid was carefully decanted from the sediment. The treatment was repeated twice, with 250 cubic centimeters of water each time. The boiling was confined to fifteen minutes. The three aqueous extracts were combined and rendered acid with a few drops of acetic acid and then boiled for a few minutes to coagulate the soluble proteins. While hot, the liquid was filtered under pressure through an ordinary filter paper supported underneath by linen. A hardened filter paper could not be used because of the mucilaginous nature of the aqueous extract. When the extract was cold, it was made up to a volume of 1,000 cubic centimeters. No preservative was used in the extract, as the experiment was so arranged that all the determinations were started the same day. The object of setting aside the powdered leaves after they were boiled with water was to allow the fine particles of the powder to settle out so that the clear mucilaginous aqueous extract could be removed by decantation. The method generally followed of filtering the aqueous extract of the leaves immediately after boiling was also tried, but was given up because the extract was so viscous that filtration was very slow.

*Water-soluble nitrogen.*—Two 50-cubic-centimeter portions of the aqueous extract were transferred to Kjeldahl digestion flasks and evaporated almost to dryness. The total nitrogen was determined in exactly the same way as in the leaf powder, except that 15 cubic centimeters of sulphuric acid, 1 gram of salicylic acid, and 2.5 grams of sodium thiosulphate were used.

*Nitrate nitrogen.*—The determination was carried out according to Scales's method as modified by Harrison.(20) The principle is the reduction of the nitrate nitrogen to ammonia in alkaline solution by means of some reducing agent and, after reduction, distillation into a known amount of acid, the excess of which is found by titration. Harrison(20) has pointed out the advantage of Scales's method over that of Devarda. Since the method was to be used for the nitrate nitrogen in plant ex-

tracts, it was necessary to determine first whether other forms of nitrogen in the extract might also be included. A series of experiments was conducted, using an extract of the sugar beet which was available at that time. Nitrogen in the form of acid amide, amino acid, nitrate, nitrite, and ammonia was added to known volumes of the extract and the amount of nitrogen recovered from each of them was determined. The results of the experiments are shown in Table 3.

TABLE 3.—*Results of nitrate nitrogen determination.*

| | N/14 $H_2SO_4$ consumed. | Nitrogen recovered. | Nitrogen present. | Nitrogen recovered. |
|---|---|---|---|---|
| | *cc.* | *mg.* | *mg.* | *Per cent.* |
| 50 cc. of extract | 48.90 | | | |
| 50 cc. extract + 0.625 gm. sugar | 48.87 | | | |
| 50 cc. extract + 0.25 gm. asparagine + 0.1992 gm. acetanilide | 50.90 | 2.00 | 73.60 | 2.71 |
| 50 cc. extract + 0.25 gm. asparagine + 0.625 gm. sugar | 50.30 | 1.40 | 53.00 | 2.64 |
| 50 cc. of extract + 0.25 gm. asparagine | 50.70 | 1.80 | 53.00 | 3.39 |
| 50 cc. extract + 0.625 gm. sugar + 0.40 gm. alanine | 48.70 | 0.00 | 62.90 | 0.00 |
| 50 cc. extract + 0.075 gm. $KNO_3$ | 59.10 | 10.20 | 10.38 | 98.26 |
| 50 cc. extract + 0.0375 gm. $KNO_3$ | 54.20 | 5.30 | 5.19 | 102.11 |
| 200 cc. distilled water + 0.2038 gm. $KNO_3$ | 28.30 | 28.30 | 28.20 | 100.35 |
| 200 cc. distilled water + 0.3247 gm. $NaNO_2$ | 64.30 | 64.30 | 65.91 | 97.55 |
| 200 cc. distilled water + 0.1179 gm. $NH_4NO_3$ | 39.30 | 39.30 | 40.12 | 97.95 |
| 50 cc. extract + 0.05895 gm. $NH_4NO_3$ | 68.62 | 19.72 | 20.06 | 98.60 |

The results indicate that only a small percentage of the nitrogen from acid amides, as in acetanilide, is determined as nitrate by this method. The amino acid nitrogen, as in alanine, remains unaffected. The same holds true, of course, for the two types of nitrogen in asparagine. As was expected, the method accounts for all the nitrogen from nitrites and ammonia—a fact which must be taken into account in using the method. In *Oenothera* leaves, no nitrite was found, so the nitrate nitrogen as determined by this method includes only the ammonia nitrogen. Since the latter is also known, the nitrate nitrogen can be found by difference. The method as adopted was therefore as follows: Two 200-cubic-centimeter portions of the aqueous extract were transferred to 500-cubic-centimeter Kjeldahl digestion flasks which contained 80 grams of zinc-copper couple. Then 6.5 grams of sodium chloride magnesium oxide mixture (5 grams of sodium chloride to 1.5 grams of magnesium oxide) were added to the contents of the flasks and the distillation carried

out. The distillate was collected in a known amount of 0.1 *N* sulphuric acid, until 150 cubic centimeters of the distillate were obtained. The excess of acid was determined by titration. A blank experiment, using 200 cubic centimeters of distilled water, was also carried out.

The acid amide, humin, and basic and nonbasic nitrogen of the aqueous extracts were determined according to the method of Hausmann as adopted by Jodidi.(22)

*Peptide nitrogen.*—A 50-cubic-centimeter sample of the aqueous extract was treated with 10 cubic centimeters of concentrated hydrochloric acid and kept boiling under a reflux condenser for three hours. The acidified extract was evaporated to dryness on the water bath to drive off the acid, and the residue was treated with a small amount of water and filtered. The filtrate was evaporated to about 10 cubic centimeters on the water bath and the nitrogen of the amino acids determined according to Van Slyke's method.(56) The nitrogen found comes from the free amino acids present in the leaves and from the amino acids formed by the breaking down of the peptide linkings by hydrolysis. To obtain the peptide nitrogen, the amino acid nitrogen found before hydrolysis was subtracted from that found after hydrolysis.

The nitrogen partition in the leaves after acid hydrolysis, was carried out according to Jodidi and Moulton's procedure(23) as applied to spinach.

### THE CARBOHYDRATES

*Sugar.*—In the determination of the sugar content of the leaves, effort was first directed to finding an appropriate process of extraction. The cold-water method was first tried but was abandoned because, owing to the slimy nature of the aqueous extract, the leaves could not be extracted repeatedly with cold water; in the second extraction, the extract would hardly pass through the filter. The same difficulty was met when hot water was tried. The use of a dilute solution of alcohol was therefore resorted to. This was found to be a satisfactory solvent. Accordingly, a series of experiments was carried out to determine the strength of alcohol to be used and the length of time required for extraction. Three different strengths of alcohol were tried, and the samples were extracted three successive times. The period of shaking for each extraction was two hours for the first and second series and one hour forty-five minutes for the third and fourth. In the fourth series, in which 10 per cent alcohol was used, it was found that the concentration was not high

enough to prevent the extract from becoming slimy. In all cases the alcoholic extract was made up to a definite volume, and the reducing sugar in an aliquot portion of the clarified solution was determined before and after inversion. The reducing sugar was determined by means of Fehling's solution, the precipitation being carried out according to the directions of Munson and Walker,(26) and the copper in the precipitate estimated according to the iodometric method of Low.(26) The results obtained are given in Table 4.

TABLE 4.—*Results of sugar extraction under various conditions.*

| Sample No. | Time of shaking. | | Alcohol. | Reducing sugar before inversion. | Reducing sugar after inversion. | Reducing sugar freed by hydrolysis. |
|---|---|---|---|---|---|---|
| | *Hrs.* | *min.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* |
| 0842—1 | 6 | | 25 | 7.42 | 10.36 | 2.94 |
| 0842—2 | 6 | | 25 | 7.46 | 10.38 | 2.92 |
| 0842—3 | 6 | | 15 | 7.41 | 10.30 | 2.89 |
| 0842—4 | 6 | | 15 | 7.44 | 10.32 | 2.88 |
| 0842—5 | 1 | 45 | 15 | 7.48 | 10.38 | 2.90 |
| 0842—6 | 1 | 45 | 15 | 7.44 | 10.37 | 2.93 |
| 0842—7 | 1 | 45 | 15 | 7.34 | 10.36 | 3.02 |
| 0842—mean | | | | 7.43 | 10.35 | 2.92 |

Since the reducing sugar obtained in all series was almost the same, there was no advantage in using a 25 per cent alcohol, nor in allowing the extraction to proceed for a long period of time. To insure perfect extraction of the sugar, it is of course preferable to use a low concentration of alcohol. Since a 10 per cent strength removed some mucilage and was therefore unsatisfactory, the 15 per cent alcohol was chosen for further work.

Since in the previous experiments no precautions were taken to destroy or to inhibit the action of the enzymes present in the leaves, the results were compared with those obtained by using a very strong solution of alcohol as a solvent. A known weight of the sample was placed in a Soxhlet extractor and extracted first with anhydrous ether to remove the chlorophyll and other, fatty substances. The powder was dried and then extracted with 95 per cent alcohol for forty-eight hours. The alcohol of the extract was recovered by distillation under reduced pressure, and the residue was dissolved in water and made up to a definite volume. The reducing sugar in the clarified solution of an aliquot portion of the extract was determined in the same way as in the previous experiments. The residual leaves were dried and extracted with water, to remove the sugar which was not

dissolved by the alcohol, and the reducing sugar in the aqueous extract was determined. The results obtained are shown in Table 5.

TABLE 5.—*Comparison of analytical results for sugar with and without taking precautions for the inactivation of enzymes.*

| | Sample No. | Reducing sugar before inversion. | Reducing sugar after inversion. | Reducing sugar freed by hydrolysis. |
|---|---|---|---|---|
| | | *Per cent.* | *Per cent.* | *Per cent.* |
| Ninety-five per cent alcoholic extract | 0842–A | 5.66 | 7.84 | 2.18 |
| Aqueous extract | 0842–A | 1.91 | 2.66 | 0.75 |
| Total sugar; enzymes inactivated | 0842–A | 7.57 | 10.50 | 2.93 |
| Ninety-five per cent alcoholic extract | 0842–B | 6.48 | 8.97 | 2.49 |
| Aqueous extract | 0842–B | 1.08 | 1.44 | 0.36 |
| Total sugar; enzymes inactivated | 0842–B | 7.56 | 10.41 | 2.85 |
| Mean results of total sugar; enzymes inactivated | 0842 | 7.57 | 10.46 | 2.89 |
| Mean results from Table 4; enzymes not inactivated | 0842 | 7.43 | 10.36 | 2.92 |

The sugars found by using strong hot alcohol, to prevent the action of the enzymes, were practically in the same proportions as when weak alcoholic solutions were used. Only a very small portion of the sugars in *Oenothera* leaves is present as non-reducing sugar; hence the enzymatic effect was not appreciable. Furthermore, the presence of even a low concentration of alcohol might possibly retard the enzymatic action.

Since the ordinary method of extraction with a weak solution of alcohol is rapid and yields the same results as more complicated procedures, the method adopted for extraction of the sugars was as follows: A 5-gram sample was transferred to an Erlenmeyer flask, 250 cubic centimeters of 15 per cent alcohol added, and the flask shaken in a shaking machine for one hour. The extract was filtered by suction. The powder with the filter paper was returned to the original flask, 150 cubic centimeters of the alcoholic solution were added, and the flask was shaken for thirty minutes. The extract was filtered and the process repeated, using 100 cubic centimeters of the alcoholic solution and shaking for fifteen minutes. The combined alcoholic extracts were made up to a volume of 500 cubic centimeters. An aliquot portion of the extract was at once withdrawn, clarified with lead subacetate solution, and the excess lead removed with sodium carbonate. The reducing sugar in the clarified solution before and after inversion was then determined according to the method already described.

*Pentosan.*—The pentosan was determined in a sample of the dried leaves according to the method adopted by the Association of Official Agricultural Chemists for pentosans in foods and feeding stuffs.(26)

*Starch.*—In *Oenothera* leaves tannins occur in such considerable amounts that they must be removed before the starch can be determined by the diastase method with subsequent acid hydrolysis. Accordingly, a given amount of the powdered leaves was extracted in a Soxhlet extractor with 95 per cent alcohol for forty-eight hours. The powder was allowed to dry and then treated with cold water to remove any residual tannin that is insoluble in 95 per cent alcohol. The powder after undergoing this treatment was transferred to a beaker, 50 cubic centimeters of water added, and the starch determined according to the directions given in the Journal of the Association of Official Agricultural Chemists,(26) except that the dextrose resulting from the hydrolysis of starch was estimated according to the method adopted for the free reducing sugar.

*Tannin.*—The tannin was determined according to the Proctor modification of the Löwenthal method as applied to tea by the Association of Official Agricultural Chemists.(26) In *Oenothera* leaves, which give a mucilaginous aqueous extract, it was found best to set aside the leaf infusion overnight, after which the supernatant liquid could be decanted.

*Crude fiber.*—A 2-gram sample of the leaves was extracted with ordinary ether in a Soxhlet extractor until the percolate was colorless. The leaves were dried and the crude fiber determined according to the directions given in the Journal of the Association of Official Agricultural Chemists for crude fiber in foods and feeding stuffs.(26)

*The water-soluble acids.*—A 5-gram sample of the leaves was transferred to an Erlenmeyer flask, 200 cubic centimeters of distilled water added, and the whole shaken for thirty minutes. The extract was filtered by suction. A 20-cubic-centimeter aliquot portion of the filtrate was diluted with 100 cubic centimeters of distilled water and titrated with 0.1 *N* sodium hydroxide using phenolphthalein as an indicator. The total acids found were expressed in terms of the number of milligrams of sodium hydroxide required to neutralize the acids from one gram of moisture-free sample.

*Total ash.*—A sample, weighing 2 to 3 grams, was incinerated in a platinum crucible at a low heat until it was reduced to a gray ash. It was cooled, treated with hot water to dissolve the

soluble salts, and filtered. The filter paper with the carbonaceous residue was transferred to the original crucible, dried, and heated to full redness until the ash was white. The filtrate was evaporated to a small volume on the water bath and the concentrated solution was added a little at a time to the crucible containing the insoluble ash and the whole evaporated to dryness on the water bath. A few cubic centimeters of strong ammonium carbonate were added in order to change the alkaline earth oxides into their carbonates. The crucible was then heated in a free flame to a very dull red heat until a constant weight was obtained. The ash is reported as containing carbonates.

### THE INORGANIC CONSTITUENTS OF THE ASH

A 30-gram sample of the leaves was incinerated at a low heat in a porcelain dish until a gray ash was obtained. The ash was then treated in the same way as in the determination of the total ash. In this case, of course, a large platinum dish was used. The ash obtained was pulverized and kept in a well-stoppered bottle. An aliquot portion of the ash, weighing from 2.5 to 3 grams, was treated with 200 cubic centimeters of 10 per cent hydrochloric acid and the solution evaporated to complete dryness on the water bath in order to dehydrate the silica. The residue was taken up with 100 cubic centimeters of 10 per cent hydrochloric acid and 200 cubic centimeters of warm water. It was filtered, washed thoroughly with water, and the filtrate after cooling was made up to 500 cubic centimeters. In the subsequent determinations, this filtrate was referred to as the original solution.

*Silica.*—The residue obtained in the preparation of the solution of the ash was treated according to directions given in the official methods of the Association of Official Agricultural Chemists for silica in plants.(26) The silica was estimated by the loss of weight of the residue when treated with hydrofluoric acid.

*Calcium.*—A 100-cubic-centimeter sample of the original solution was taken and the calcium precipitated as oxalate according to directions given by Mitchell.(32) The calcium oxalate was dissolved in hot 10 per cent sulphuric acid and at once titrated with 0.1 *N* potassium permanganate.

*Magnesium.*—To the filtrate and washings from the calcium determination, 25 cubic centimeters of concentrated nitric acid were added and the acid liquid was evaporated to complete dryness on the water bath. The residue was taken up with dilute

hydrochloric acid and the magnesium was precipitated as magnesium ammonium phosphate according to the method given by Mitchell.(32) The precipitate was ignited, and weighed as magnesium pyrophosphate.

*Phosphoric acid.*—The phosphoric acid was determined in 50 cubic centimeters of the original solution by one of the methods given in the Journal of the Association of Official Agricultural Chemists,(26) in which the phosphoric acid was precipitated as ammonium phosphomolybdate, and the precipitate was dissolved in a known excess of 0.1 *N* sodium hydroxide, the excess alkali being determined by titration.

*Sulphuric acid.*—The determination was carried out according to directions given in the official methods of the Association of Official Agricultural Chemists, 100 cubic centimeters of the original solution being used.

*Potassium and sodium.*—The filtrate and washings from the sulphuric acid determination were treated according to the directions given in the official methods of the Association of Official Agricultural Chemists(26) for potassium and sodium in plant products. When the potassium and sodium chlorides were obtained, the separation of potassium was accomplished by the perchlorate method, following in detail the directions given by Willard.(52)

### PRESENTATION OF RESULTS

In all the tables the results are the mean of two determinations which agreed with each other within the limit of experimental error. Unless otherwise stated, the results are expressed as percentages of the moisture-free samples. In the list of the strains studied and in the tabulation of results, the pistillate parent is named first and then the pollen parent in all strains which resulted from crosses.

### THE NITROGEN DISTRIBUTION

The chief nitrogenous compounds from the point of view of their importance in the life processes of the plant are the proteins, amino acids, peptides, acid amides, nitrates, and some of the nitrogen bases. The nitrogen source for the parasitic fungi is of course the body of the host. The fungi may conceivably obtain their nitrogen either by direct absorption of the soluble simpler nitrogenous compounds or by the digestion of colloidal compounds, which are, as far as known, proteins.

The major portion of the nitrogenous constituents of the plant is protein which, at least as far as storage protein is concerned, is continually being decomposed into amino-acid units and again resynthesized. In a diseased plant, the fungus must disturb the normal nitrogen distribution, even if it uses only amino acids produced in a normal manner by the host, whether by primary synthesis or by autodigestion of protein. Disturbance might be even greater if the fungus excreted proteases which digested the plant proteins independently of the normal metabolism of the host. It is, therefore, a logical expectation that the nitrogen distribution in healthy plants will not be the same as it is in diseased plants.

Among the first investigators to find significant differences in the nitrogen distribution between healthy and diseased plants was Jodidi.(22) He studied the nitrogenous constituents of healthy and mosaic cabbage, and with Moulton and Markley(24) extended his investigation to the mosaic disease of spinach. In both plants the diseased individuals were found to have a lower percentage of total nitrogen, acid amide, monoamino and diamino-nitrogen than the normal ones. Moreover, nitrous acid was present only in the diseased plants. These characteristic features of the diseased cabbage and spinach were ascribed to denitrification in the affected tissues, by which nitrates were reduced in part to ammonia and in part to nitrites. The latter reacting with the amino groups of the various organic compounds brought about the elimination of elementary nitrogen. There is room for doubt as to the correctness of Jodidi's explanation. However, his results show conclusively that in mosaic plants the nitrogen distribution is different from that in normal ones. Since no causal organism has been demonstrated for the mosaic diseases, there was no reason to look for findings parallel to Jodidi's in the present investigation of mildewed *Oenothera*. The results are, in fact, very diverse. As far as I know, no previous investigation has dealt in a thoroughgoing way with a known fungus infection from the standpoint of nitrogen distribution.

*Total nitrogen.*—In *Oenothera*, there exists a remarkable difference in the proportion of the various groups of nitrogen compounds in the resistant and susceptible strains. First of all, let us consider the results obtained for total nitrogen, which are shown in Table 6.

TABLE 6.—*Percentage of total nitrogen in the leaves of different strains of Oenothera.*

| Key No. | Name of strain. | Characteristic of strain. | Nitrogen in 1920. | Nitrogen in 1921. | Nitrogen in 1922. |
|---|---|---|---|---|---|
| | | | *Per cent.* | *Per cent.* | *Per cent.* |
| 1 | Resistant *Oe. pratincola* (a segregate from species hybrid). | Resistant | 2.55 | | |
| 1a | | | | 2.59 | |
| 1b | | | | | 2.54 |
| 2 | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 3.39 | | |
| 2a | | | | 3.61 | |
| 2b | | | | | 3.03 |
| 3 | *Oe. pratincola* hyb. *immunis* | Resistant | 2.59 | | |
| 3a | | | | 2.79 | |
| 3b | | | | | 2.29 |
| 4 | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 3.55 | | |
| 4a | | | | 3.87 | |
| 4b | | | | | 3.08 |
| 5 | *Oe. mississippiensis* × *Oe. pratincola* hyb. *immunis* | Resistant | 3.03 | | |
| 5a | | | | 3.25 | |
| 6 | *Oe. pratincola* hyb. *immunis* × *Oe. mississippiensis* | Susceptible | 3.38 | | |
| 6a | | | | 3.57 | |
| 7 | *Oe. mississipiensis* × *Oe. pratincola* hyb. *immunis* | Resistant | 3.00 | | |
| 8 | *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis* | do | | 3.22 | |
| 8a | | | | | 2.45 |
| 9 | *Oe. pratincola* hyb. *immunis* × *Oe. cinerescens* | Susceptible | | 3.65 | |
| 9a | | | | | 2.81 |
| 10 | *Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *immunis* | Resistant | 3.13 | | |
| 11 | *Oe. pratincola* hyb. *immunis* × *Oe. "biennis Chicago"* | Susceptible | 3.62 | | |
| 12 | *Oe. cinerescens* × *Oe. mississippiensis* | Resistant | 2.64 | | |
| 13 | *Oe. mississippiensis* × *Oe. cinerescens* | Susceptible | 2.93 | | |
| 13a | | | | 2.93 | |
| 13b | | | | | 2.69 |
| 14 | *Oe. cinerescens* × *Oe. mississippiensis* (metaclinic hybrid) | do | | 2.86 | |
| 14a | | | | | 2.64 |
| 15 | *Oe. pratincola* × *Oe. reynoldsii* (*Oe. pratincola* hyb. *amycosa*). | Resistant | 2.22 | | |
| 16 | *Oe. reynoldsii* × *Oe. pratincola* | Susceptible | 2.71 | | |
| 17 | *Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx* | do | 3.13 | | |
| 17a | | | | 2.93 | |
| 17b | | | | | 2.68 |
| 18 | *Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago"* | do | 3.74 | | |
| 18a | | | | 3.16 | |
| 18b | | | | | 2.81 |
| 19 | *Oe. mississippiensis* × *Oe. "biennis Chicago"* | do | 2.88 | | |
| 20 | *Oe. "biennis Chicago"* × *Oe. mississippiensis* | do | 3.25 | | |
| 21 | *Oe. "biennis Chicago"* × *Oe. mississippiensis* | do | 3.08 | | |

TABLE 6.—*Percentage of total nitrogen in the leaves of different strains of Oenothera*—Continued.

| Key No. | Name of strain. | Characteristic of strain. | Nitrogen in 1920. | Nitrogen in 1921. | Nitrogen in 1922. |
|---|---|---|---|---|---|
| | | | *Per cent.* | *Per cent.* | *Per cent.* |
| 24 | *Oe. pratincola* mut. *nitidissima* | Resistant | | 2.79 | |
| 24a | do | do | | 2.92 | |
| 25 | do | Susceptible | | 3.23 | |
| 25a | do | do | | 3.16 | |
| 26 | *Oe. numismatica* | Resistant | | 2.40 | |
| 27 | *Oe. pratincola* mut. *simulans* | do | | 2.34 | |
| 29a | *Oe. pratincola* hyb. *immunis* | do | | | 2.54 |
| 33 | *Oe. reynoldsii* | do | | 2.28 | |
| 34 | *Oe. pratincola* hyb. *rubricalyx* | Susceptible | | | 2.95 |
| 35 | *Oe. "biennis Chicago"* | do | | | 2.91 |

(*a*) For all pairs of reciprocal hybrids, the susceptible members of the pairs contain a higher percentage of total nitrogen than do the resistant ones. This difference persisted in the strains grown for three consecutive years.

(*b*) The same statement holds true for elementary species. The resistant species are found to have less total nitrogen than the susceptible species.

(*c*) In *Oenothera pratincola* mut. *nitidissima* (key Nos. 24–25), which occurs in susceptible and resistant forms, the susceptible form has a higher total nitrogen than the resistant one. This mutation, as has been stated elsewhere in this paper, has been recovered in many progenies of crosses into which it entered, unchanged except for modification of its susceptibility.

(*d*) Pairs of strains which have the same genetic composition but belong to different pedigrees, contain almost the same percentage of total nitrogen. This fact is shown in the crosses *Oe. mississippiensis* × *Oe. pratincola* hyb. *immunis* (key Nos. 5 and 7) and *Oe. mississippiensis* × *Oe. "biennis Chicago"* (key nos. 19 and 21).

(*e*) A striking situation is provided by the reciprocal hybrids from crosses between *Oe. cinerescens* (immune) and *Oe. mississippiensis* (susceptible). The hybrid (key No. 12), in which *Oe. cinerescens* is the pistillate parent, is resistant and therefore has a lower total nitrogen than the reciprocal hybrid *Oe. mississippiensis* × *Oe. cinerescens* which is susceptible (key No. 13). But the metaclinic hybrid *Oe. cinerescens* × *Oe. mississippiensis* (key No. 14), being of the type *Oe. mississippiensis*, is susceptible. We should expect it to show a higher total nitrogen than the resistant matroclinic plants (key No. 12) and this is exactly the case, for the percentage of total nitrogen found is almost the

same as in its reciprocal hybrid, where *Oe. mississippiensis* is the pistillate parent (key No. 13). The two were grown for two successive years, and the difference in their total nitrogen is very slight. The data for the $F_2$ generation of the resistant hybrid are lacking, as the self-pollinated matroclinic plant of the $F_1$ gave no seeds. Genetical data regarding this metaclinic hybrid are given by Klaphaak and Bartlett.(27)

(*f*) In pairs of reciprocal hybrids in which both members are susceptible, the total nitrogen is comparatively high and the difference between reciprocals is slight. The only exception is found in the results for 1920 of the crosses between *Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx*, where the variation in total nitrogen is rather high. But the same reciprocal hybrids, when grown in 1921 and 1922, show much less difference. Since the collection for 1920 was made when the plants were approaching senescence, presumably the cause of the variation is traceable to this fact. Hybrids of this type are shown in Table 6 under key Nos. 17 and 18 and Nos. 19 and 20.

(*g*) In crosses between resistant and susceptible parents, the resistant hybrid and the resistant parent exhibit a low percentage of total nitrogen. The reverse is true for the susceptible members, which both have high total nitrogen. Thus, resistant *Oe. pratincola* (key No. 1), a segregate from a species hybrid, and its reciprocal hybrid, mildewed *Oe. pratincola* (key No. 2), come from *Oe. pratincola* hyb. *immunis* (key No. 29a) and *Oe. pratincola* hyb. *rubricalyx* (key No. 34). Resistant *Oe. pratincola,* according to Klaphaak and Bartlett,(27) has the zygotic composition $\alpha\hat{\imath}\beta$I and hence it is immune. (The formation takes account only of the immunity factor.) Its resistant parent, *Oe. pratincola* hyb. *immunis,* has also the zygotic composition $\alpha\hat{\imath}\beta$I. Both of them have 2.54 per cent total nitrogen. The mildewed *Oe. pratincola* has also the same zygotic composition as its susceptible parent, *Oe. pratincola* hyb. *rubricalyx,* which is $\alpha\hat{\imath}\beta_\iota$, and their total nitrogen varies only very slightly. The hybrid has 3.03 per cent and the parent has 2.95 per cent.

(*h*) In crosses between two susceptible parents, the resultant hybrids, which are both susceptible, agree rather closely with their parents in having a proportionately high total nitrogen. This fact is illustrated by crosses between *Oe. pratincola* hyb. *rubricalyx* and *Oe. "biennis Chicago"* (key Nos. 17b, 18b, 34, and 35).

TABLE 7.—*Nitrogen partition in the leaves of the different strains of Oenothera.*

| Key No. | Name of strain. | Characteristic of strain. | Total nitrogen. | Water-soluble nitrogen. | Protein nitrogen. | Free amino-acid nitrogen. | Nitrate nitrogen. | Ammonia nitrogen. | Water-soluble nitrogen. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Acid amide and ammonia nitrogen. | Humin nitrogen. | Basic nitrogen. | Non-basic nitrogen. | Peptide nitrogen. |
| | | | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* |
| 1b | Resistant *Oe. pratincola* (a segregate from species hybrid). | Resistant | 2.54 | 0.427 | 2.09 | 0.235 | 0.069 | 0.055 | 0.162 | 0.151 | 0.092 | 0.022 | 0.106 |
| 2b | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 3.03 | 0.607 | 2.46 | 0.311 | 0.087 | 0.076 | 0.127 | 0.188 | 0.121 | 0.171 | 0.020 |
| 3b | *Oe. pratincola* hyb. *immunis* | Resistant | 2.29 | 0.709 | 1.55 | 0.421 | 0.132 | 0.105 | 0.254 | 0.194 | 0.109 | 0.152 | 0.00 |
| 4b | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 3.08 | 1.06 | 2.03 | 0.588 | 0.188 | 0.137 | 0.327 | 0.219 | 0.107 | 0.407 | 0.067 |
| 8a | *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*. | Resistant | 2.45 | 0.575 | 1.97 | 0.351 | 0.138 | 0.031 | 0.168 | 0.185 | 0.095 | 0.127 | 0.056 |
| 9a | *Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*. | Susceptible | 2.81 | 0.848 | 1.99 | 0.446 | 0.154 | 0.037 | 0.213 | 0.188 | 0.118 | 0.329 | 0.00 |
| 17b | *Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx*. | do | 2.68 | 0.667 | 2.08 | 0.537 | 0.157 | 0.093 | 0.218 | 0.177 | 0.091 | 0.181 | 0.014 |
| 18b | *Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago."* | do | 2.81 | 0.757 | 2.11 | 0.433 | 0.147 | 0.093 | 0.244 | 0.169 | 0.112 | 0.232 | 0.025 |
| 29a | *Oe. pratincola* hyb. *immunis* | Resistant | 2.54 | 0.740 | 1.82 | 0.482 | 0.115 | 0.098 | 0.215 | 0.152 | 0.071 | 0.301 | 0.00 |
| 34 | *Oe. pratincola* hyb. *rubricalyx* | Susceptible | 2.95 | 0.933 | 2.09 | 0.739 | 0.166 | 0.117 | 0.277 | 0.193 | 0.098 | 0.365 | 0.00 |
| 35 | *Oe. "biennis Chicago"* | do | 2.91 | 1.06 | 2.00 | 0.626 | 0.190 | 0.116 | 0.318 | 0.123 | 0.127 | 0.492 | 0.133 |

In view of the concordant results obtained from all the possible crosses and elementary species, it can be stated that susceptible plants are characterized by having a higher total nitrogen than closely related resistant ones.

*Water-soluble nitrogen.*—To determine which forms of nitrogen are responsible for the difference in total nitrogen, the nitrogen distribution was determined for four pairs of reciprocal hybrids and three elementary species. It is to be noted that the figures for acid amide and ammonia, humin, and basic and nonbasic nitrogen, given in Tables 7 and 8, were obtained after acid hydrolysis of the water-soluble extract, hence they account for the nitrogen from the water-soluble portion only. Results in Table 7 indicate that: (*a*) in the three pairs of reciprocal hybrids which consist of resistant and susceptible members (key Nos. 1b–9a), the water-soluble nitrogen as a whole and its amino acid and nonbasic nitrogen compounds are higher in the susceptible hybrids than in their resistant reciprocals. The nitrate nitrogen in the susceptible hybrids is somewhat higher than in the resistant ones but the difference is not sufficient to be considered significant. As for the protein nitrogen, in the first two pairs (key Nos. 1b–4b) the susceptible hybrids contain higher protein nitrogen than the resistant ones, but in the third pair (key Nos. 8a–9a) their protein nitrogen is about the same. Other forms of nitrogen do not show concordant results. (*b*) In the fourth pair of reciprocal hybrids, in which both members are susceptible (key Nos. 17b–18b), the water-soluble nitrogen is high and the percentage of the different forms of nitrogen in the water-soluble fraction of both strains does not vary considerably. (*c*) The resistant parent shows the same characteristic features, when compared with the susceptible parent, as its resistant offspring; namely, low water-soluble nitrogen, amino acid, and nonbasic nitrogen. Conversely, the susceptible parent and its susceptible progeny resemble each other in having high water-soluble nitrogen, amino acid, and nonbasic nitrogen.

When the results of the nitrogen distribution, shown in Table 7, are expressed as percentage of the total nitrogen of the leaves, it can be seen that the water-soluble nitrogen is also higher in the susceptible strains than in the resistant ones. The two main components of the water-soluble nitrogen which account for this difference are the free amino acid and the nonbasic nitrogen. For the protein nitrogen, the reverse is found to be the case. (See Table 8.)

TABLE 8.—*Nitrogen partition in the leaves of the different strains of Oenothera.*

[Data expressed as percentage of the total nitrogen of the leaves.]

| Key No. | Name of strain. | Characteristic of strain. | Water-soluble nitrogen. | Protein nitrogen. | Free amino-acid nitrogen. | Nitrate nitrogen. | Ammonia nitrogen. | Water-soluble nitrogen. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Acid amide and ammonia nitrogen | Humin nitrogen. | Basic nitrogen. | Non-basic nitrogen. | Peptide nitrogen. |
| 1b | Resistant *Oe. pratincola* (a segregate from species hybrid). | Resistant | 16.81 | 82.28 | 9.25 | 2.72 | 2.17 | 6.38 | 5.94 | 3.62 | 0.866 | 4.17 |
| 2b | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 20.03 | 81.19 | 10.26 | 2.87 | 2.51 | 4.19 | 6.21 | 3.99 | 5.64 | 0.660 |
| 8a | *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*. | Resistant | 23.47 | 80.41 | 14.33 | 5.63 | 1.27 | 6.86 | 7.55 | 3.88 | 5.18 | 2.29 |
| 9a | *Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*. | Susceptible | 30.18 | 70.82 | 15.87 | 5.48 | 1.32 | 7.58 | 6.69 | 4.20 | 11.71 | 0.00 |
| 3b | *Oe pratincola* hyb. *immunis* | Resistant | 30.96 | 67.69 | 18.39 | 5.76 | 4.59 | 11.09 | 8.47 | 4.76 | 6.64 | 0.00 |
| 4b | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 34.42 | 65.94 | 19.09 | 6.10 | 4.45 | 10.79 | 7.11 | 3.47 | 13.21 | 2.18 |
| 17b | *Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx*. | do | 24.89 | 77.61 | 10.04 | 5.86 | 3.47 | 8.13 | 6.60 | 3.40 | 6.75 | 0.522 |
| 18b | *Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago"*. | do | 26.94 | 76.09 | 15.41 | 5.23 | 3.31 | 8.68 | 6.01 | 3.99 | 8.26 | 0.889 |
| 29a | *Oe. pratincola* hyb. *immunis* | Resistant | 29.13 | 71.65 | 18.98 | 4.53 | 3.86 | 8.50 | 5.98 | 2.80 | 11.85 | 0.00 |
| 34 | *Oe. pratincola* hyb. *rubricalyx* | Susceptible | 31.63 | 70.85 | 25.05 | 5.63 | 3.97 | 9.39 | 6.54 | 3.32 | 12.37 | 0.00 |
| 35 | *Oe. "biennis Chicago"* | do | 36.43 | 68.73 | 21.51 | 6.53 | 3.99 | 10.93 | 4.23 | 4.36 | 16.91 | 4.57 |

*Protein nitrogen.*—In order to determine the nitrogen partition in the protein, the leaf powder was treated with strong hydrochloric acid and boiled under a reflux condenser for several hours. After hydrolysis was complete, the nitrogen distribution was determined, according to the methods of Hausmann as adopted by Jodidi and Moulton(23) in their work with spinach. The results of the experiments are shown in Tables 9 and 10.

TABLE 9.—*Nitrogen partition in the leaves of different strains of Oenothera after acid hydrolysis.*

| Key No. | Name of strain. | Characteristic of strain. | Acid amide and ammonia nitrogen. | Humin nitrogen. | Basic nitrogen. | Non-basic nitrogen. |
|---|---|---|---|---|---|---|
| | | | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* |
| 1b | Resistant *Oe. pratincola* (a segregate from species hybrid). | Resistant | 0.254 | 0.237 | 0.398 | 1.651 |
| 2b | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 0.370 | 0.230 | 0.499 | 1.931 |
| 3b | *Oe. pratincola* hyb. *immunis* | Resistant | 0.384 | 0.218 | 0.334 | 1.354 |
| 4b | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 0.556 | 0.249 | 0.476 | 1.799 |
| 8a | *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*. | Resistant | 0.307 | 0.226 | 0.379 | 1.538 |
| 9a | *Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*. | Susceptible | 0.372 | 0.213 | 0.397 | 1.828 |
| 13b | *Oe. mississippiensis* × *Oe. cinerescens*. | do | 0.467 | 0.251 | 0.379 | 1.593 |
| 14b | *Oe. cinerescens* × *Oe. mississippiensis* (metaclinic hybrid). | do | 0.466 | 0.196 | 0.360 | 1.618 |
| 17b | *Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx*. | do | 0.404 | 0.257 | 0.404 | 1.615 |
| 18b | *Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago"*. | do | 0.371 | 0.258 | 0.417 | 1.764 |
| 29a | *Oe. pratincola* hyb. *immunis* | Resistant | 0.342 | 0.180 | 0.436 | 1.582 |
| 34 | *Oe. pratincola* hyb. *rubricalyx* | Susceptible | 0.465 | 0.255 | 0.489 | 1.741 |
| 35 | *Oe. "biennis Chicago"* | do | 0.434 | 0.160 | 0.525 | 1.791 |

Table 9 shows that, in the first two pairs of reciprocal hybrids (key Nos. 1b–4b), the acid amide and ammonia nitrogen and the basic nitrogen are higher in the susceptible hybrids than in their resistant reciprocals. In the third pair (key Nos. 8a–9a), however, this difference is not shown to a great extent. With respect to the nonbasic nitrogen, the susceptible hybrids were found to have a higher percentage. The case of the fourth pair, where *Oe. mississippiensis* and *Oe. cinerescens* are the parents (key Nos. 13b–14b), is interesting. One of the members of the pair is a metaclinic hybrid and, as far as

their reaction to mildew is concerned, both are represented as having the same zygotic composition. Their total nitrogen has already been found to be practically the same (Table 6) and here the nitrogen distribution differs to but a slight degree. The fifth pair of reciprocal hybrids (key Nos. 17b and 18b), both members being susceptible, have high acid amide and ammonia nitrogen, and basic and nonbasic nitrogen. Furthermore, the difference between them is slight. Of the parent species (key Nos. 29a-35), the susceptible ones have higher acid amide and ammonia, basic, and nonbasic nitrogen than the resistant one.

TABLE 10.—*Nitrogen partition in the leaves of different strains of Oenothera after acid hydrolysis.*

[Data expressed as percentage of the total nitrogen of the leaves.]

| Key No. | Name of strain. | Characteristic of strain. | Acid amide and ammonia nitrogen. | Humin nitrogen. | Basic nitrogen. | Nonbasic nitrogen. |
|---|---|---|---|---|---|---|
| 1b | Resistant *Oe. pratincola* (a segregate from species hybrid). | Resistant | 10.00 | 9.33 | 15.67 | 65.00 |
| 2b | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 12.21 | 7.59 | 16.47 | 63.73 |
| 3b | *Oe. pratincola* hyb. *immunis* | Resistant | 16.77 | 9.52 | 14.59 | 59.12 |
| 4b | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 18.05 | 8.08 | 15.45 | 58.40 |
| 8a | *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*. | Resistant | 12.53 | 9.22 | 15.47 | 62.78 |
| 9a | *Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*. | Susceptible | 13.24 | 7.58 | 14.13 | 65.05 |
| 13b | *Oe. mississippiensis* × *Oe. cinerescens*. | do | 17.36 | 9.33 | 14.09 | 59.22 |
| 14b | *Oe. cinerescens* × *Oe. mississippiensis* (metaclinic hybrid). | do | 17.65 | 7.42 | 13.64 | 61.29 |
| 17b | *Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx*. | do | 14.38 | 9.15 | 14.38 | 57.47 |
| 18b | *Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago."* | do | 13.84 | 9.63 | 15.55 | 65.82 |
| 29a | *Oe. pratincola* hyb. *immunis* | Resistant | 13.46 | 7.09 | 17.17 | 62.28 |
| 34 | *Oe. pratincola* hyb. *rubricalyx* | Susceptible | 15.76 | 8.64 | 16.58 | 59.01 |
| 35 | *Oe. "biennis Chicago"* | do | 14.91 | 5.50 | 18.04 | 61.54 |

Table 10 shows that the total nitrogen of all the susceptible strains is higher than that of the resistant strains in acid amide and ammonia nitrogen. On the other hand, the total nitrogen of the resistant strains is higher in humin nitrogen than that of the susceptible ones. As for the other nitrogen groups, the results are not concordant.

The nitrogen distribution after acid hydrolysis includes both the nitrogen from the insoluble portion and from the water-soluble portion. Since the nitrogen distribution in the water-soluble portion has also been determined, the different forms of nitrogen corresponding to the water-insoluble or protein portion can be found approximately by subtraction. In the water-insoluble portion, Table 11, it can be noticed that the acid amide and ammonia nitrogen, as well as the nonbasic nitrogen, are higher in the susceptible strains than in the resistant ones. An exception occurs in *Oe. "biennis Chicago,"* one of the parent species, where the acid amide and ammonia nitrogen and the nonbasic nitrogen are somewhat low. However, when these nitrogen groups of the water-insoluble portions are expressed in percentages of the total nitrogen of the leaves, Table 12, the only significant difference that can be noticed is in the nonbasic nitrogen. With respect to this group, the resistant strains are higher than the susceptible. With the exception of *Oe. pratincola* hyb. *immunis*, a parent strain (key No. 29a), the humin nitrogen is also higher in the resistant strains.

TABLE 11.—*Nitrogen partition in the water-insoluble portion of the leaves of different strains of Oenothera, after acid hydrolysis.*

| Key No. | Name of strain. | Characteristic of strain. | Acid amide and ammonia nitrogen. | Humin nitrogen. | Basic nitrogen. | Nonbasic nitrogen. |
|---|---|---|---|---|---|---|
| | | | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* |
| 1b | Resistant *Oe. pratincola* (a segregate from species hybrid). | Resistant | 0.092 | 0.086 | 0.306 | 1.629 |
| 2b | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 0.243 | 0.042 | 0.378 | 1.760 |
| 3b | *Oe. pratincola* hyb. *immunis* | Resistant | 0.130 | 0.024 | 0.225 | 1.202 |
| 4b | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 0.229 | 0.030 | 0.369 | 1.392 |
| 8a | *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*. | Resistant | 0.139 | 0.041 | 0.284 | 1.411 |
| 9a | *Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*. | Susceptible | 0.159 | 0.025 | 0.279 | 1.499 |
| 17b | *Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx*. | do | 0.186 | 0.080 | 0.313 | 1.434 |
| 18b | *Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago."* | do | 0.127 | 0.089 | 0.305 | 1.532 |
| 29a | *Oe. pratincola* hyb. *immunis* | Resistant | 0.126 | 0.028 | 0.385 | 1.281 |
| 34 | *Oe. pratincola* hyb. *rubricalyx* | Susceptible | 0.188 | 0.062 | 0.391 | 1.376 |
| 35 | *Oe. "biennis Chicago"* | do | 0.116 | 0.037 | 0.398 | 1.299 |

TABLE 12.—*Nitrogen partition in the water-insoluble portion of the leaves of different strains of Oenothera, after acid hydrolysis.*

[Data expressed as percentage of the total nitrogen of the leaves.]

| Key No. | Name of strain. | Characteristic of strain. | Acid amide and ammonia nitrogen. | Humin nitrogen. | Basic nitrogen. | Non-basic nitrogen. |
|---|---|---|---|---|---|---|
| 1b | Resistant *Oe. pratincola* (a segregate from species hybrid). | Resistant | 3.62 | 3.39 | 12.02 | 64.13 |
| 2b | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 8.02 | 1.78 | 12.48 | 58.09 |
| 3b | *Oe. pratincola* hyb. *immunis* | Resistant | 5.68 | 1.05 | 9.83 | 52.48 |
| 4b | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 7.26 | 0.97 | 11.98 | 45.19 |
| 8a | *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*. | Resistant | 5.67 | 1.67 | 12.59 | 37.60 |
| 9a | *Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*. | Susceptible | 5.66 | 0.89 | 9.93 | 53.34 |
| 17b | *Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx*. | do | 6.25 | 2.55 | 10.98 | 50.72 |
| 18b | *Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago."* | do | 5.16 | 3.62 | 11.56 | 57.56 |
| 29a | *Oe. pratincola* hyb. *immunis* | Resistant | 4.96 | 1.11 | 14.37 | 50.43 |
| 34 | *Oe. pratincola* hyb. *rubricalyx* | Susceptible | 6.37 | 2.11 | 13.26 | 46.64 |
| 35 | *Oe. "biennis Chicago"* | do | 3.98 | 1.27 | 13.68 | 44.63 |

For the purpose of obtaining some information relative to the nitrogen distribution in the leaves of resistant and susceptible strains when they are approaching senescence and the mildew is no longer multiplying, the nitrogen distribution of three pairs of reciprocal hybrids collected in 1920 was determined. The results are shown in Tables 13 to 16. Only two concordant results are indicated in Tables 13 and 14. These are for the free amino acid nitrogen and the protein nitrogen. The resistant hybrids of the first two pairs contain more free amino acid than their corresponding reciprocals. On the other hand, the protein nitrogen of the susceptible hybrids is a great deal higher than that of the resistant ones. The difference in the free amino acid, however, loses its significance, since the last pair, in which both reciprocal hybrids are susceptible, is also found to vary in amino acid nitrogen. Therefore, the cause of the consistent difference of the total nitrogen found in the resistant and susceptible strains must be sought in the protein group. From Table 15, which shows the nitrogen distribution after acid hydrolysis, it is seen that the nitrogen from the basic and nonbasic units is higher in the susceptible strains than in the resistant. The humin nitrogen,

TABLE 13.—*Nitrogen partition in the leaves of different strains of Oenothera during the period when the leaves show signs of chlorophyll degradation (cultures of 1920).*

| Key No. | Name of strain. | Characteristic of strain. | Total nitrogen. | Water-soluble nitrogen. | Protein nitrogen. | Free amino acid nitrogen. | Ammonia nitrogen. | Nitrate nitrogen. | Water-soluble nitrogen. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Acid amide and ammonia nitrogen. | Humin nitrogen. | Basic nitrogen. | Nonbasic nitrogen. |
| 1 | Resistant *Oe. pratincola* (a segregate from species hybrid). | Resistant | 2.560 | 0.627 | 1.965 | 0.423 | 0.089 | 0.129 | 0.322 | 0.081 | 0.147 | 0.077 |
| 2 | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 3.385 | 0.389 | 3.045 | 0.263 | 0.031 | 0.080 | 0.128 | 0.070 | 0.089 | 0.096 |
| 3 | *Oe. pratincola* hyb. *immunis* | Resistant | 2.585 | 0.277 | 2.254 | 0.185 | 0.017 | 0.031 | 0.058 | 0.020 | 0.056 | 0.143 |
| 4 | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 3.547 | 0.406 | 3.150 | 0.130 | 0.044 | 0.073 | 0.092 | 0.057 | 0.071 | 0.176 |
| 17 | *Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx*. | do | 3.129 | 0.300 | 2.809 | 0.166 | 0.020 | 0.017 | 0.053 | 0.029 | 0.050 | 0.168 |
| 18 | *Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago."* | do | 3.969 | 0.741 | 3.185 | 0.467 | 0.036 | 0.081 | 0.207 | 0.032 | 0.075 | 0.427 |

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TABLE 14.—*Nitrogen partition in the leaves of different strains of Oenothera during the period when the leaves show signs of chlorophyll degradation (cultures of 1920).*

[Data expressed as percentage of the total nitrogen of the leaves.]

| Key No. | Name of strain. | Characteristic of strain. | Water-soluble nitrogen. | Protein nitrogen. | Free amino acid nitrogen. | Nitrate nitrogen. | Ammonia nitrogen. | Water-soluble nitrogen. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Acid amide and ammonia nitrogen. | Humin nitrogen. | Basic nitrogen. | Non-basic nitrogen. |
| 1 | Resistant *Oe. pratincola* (a segregate from species hybrid). | Resistant | 24.49 | 76.75 | 16.52 | 5.04 | 3.48 | 12.58 | 3.16 | 5.74 | 3.01 |
| 2 | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 11.49 | 89.95 | 7.77 | 2.36 | 0.92 | 3.78 | 2.24 | 2.63 | 2.84 |
| 3 | *Oe. pratincola* hyb. *immunis* | Resistant | 10.72 | 87.20 | 7.16 | 1.20 | 0.66 | 2.24 | 0.77 | 2.17 | 5.53 |
| 4 | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 11.45 | 88.81 | 3.67 | 2.06 | 1.24 | 2.59 | 1.61 | 2.00 | 4.96 |
| 17 | *Oe. "biennis Chicago" × Oe. pratincola* hyb. *rubricalyx.* | do | 9.59 | 89.77 | 5.31 | 0.54 | 0.64 | 1.69 | 0.93 | 1.60 | 5.36 |
| 18 | *Oe. pratincola* hyb. *rubricalyx × Oe. "biennis Chicago."* | do | 18.67 | 80.25 | 11.77 | 2.04 | 0.91 | 5.22 | 0.81 | 1.89 | 10.75 |

TABLE 15.—*Nitrogen partition in the leaves of different strains of Oenothera during the period when the leaves show signs of chlorophyll degradation (cultures of 1920).*

[After acid hydrolysis.]

| Key No. | Name of strain. | Characteristic of strain. | Acid amide nitrogen in— | | Humin nitrogen in— | | Basic nitrogen in— | | Nonbasic nitrogen in— | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Moisture-free leaves. | Total nitrogen of leaves. | Moisture-free leaves. | Total nitrogen of leaves. | Moisture-free leaves. | Total nitrogen of leaves. | Moisture-free leaves. | Total nitrogen of leaves. |
| | | | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* |
| 1 | Resistant *Oe. pratincola* (a segregate from species hybrid). | Resistant | 0.492 | 19.22 | 0.215 | 8.398 | 0.395 | 15.43 | 1.458 | 56.95 |
| 2 | Mildewed *Oe. pratincola* (a segregate from species hybrid.) | Susceptible | 0.377 | 11.14 | 0.119 | 3.516 | 0.527 | 15.57 | 2.362 | 69.78 |
| 3 | *Oe. pratincola* hyb. *immunis* | Resistant | 0.216 | 8.356 | 0.315 | 12.19 | 0.492 | 19.03 | 1.562 | 60.42 |
| 4 | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 0.339 | 9.557 | 0.189 | 5.328 | 0.722 | 20.36 | 2.297 | 64.76 |
| 17 | *Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx.* | do | 0.309 | 9.875 | 0.177 | 5.657 | 0.697 | 22.28 | 1.946 | 62.19 |
| 18 | *Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago."* | do | 0.458 | 11.54 | 0.241 | 6.072 | 0.793 | 19.98 | 2.477 | 62.41 |

TABLE 16.—*Nitrogen partition in the water-insoluble portion of the leaves of different strains of Oenothera during the period when the leaves show signs of chlorophyll degradation (cultures of 1920).*

| Key No. | Name of strain. | Characteristic of strain. | Acid amide nitrogen in— | | Humin nitrogen in— | | Basic nitrogen in— | | Nonbasic nitrogen in— | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Moisture-free leaves. | Total nitrogen of leaves. | Moisture-free leaves. | Total nitrogen of leaves. | Moisture-free leaves. | Total nitrogen of leaves. | Moisture-free leaves. | Total nitrogen of leaves. |
| | | | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* | *Per cent.* |
| 1 | Resistant *Oe. pratincola* (a segregate from species hybrid). | Resistant | 0.170 | 6.67 | 0.134 | 5.238 | 0.248 | 9.690 | 1.381 | 53.94 |
| 2 | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 0.249 | 7.36 | 0.043 | 1.276 | 0.438 | 12.940 | 2.266 | 66.94 |
| 3 | *Oe. pratincola* hyb. *immunis* | Resistant | 0.158 | 6.116 | 0.295 | 11.42 | 0.436 | 16.86 | 1.419 | 54.89 |
| 4 | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 0.247 | 6.967 | 0.132 | 3.718 | 0.651 | 18.36 | 2.121 | 59.80 |
| 17 | *Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx.* | do | 0.256 | 8.185 | 0.148 | 4.727 | 0.647 | 20.68 | 1.778 | 56.83 |
| 18 | *Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago."* | do | 0.251 | 6.32 | 0.209 | 5.262 | 0.718 | 18.09 | 2.050 | 51.65 |

however, is higher in the resistant hybrids than in their corresponding susceptible reciprocals. These differences are not shown by the last pair, where both reciprocal hybrids are susceptible. Furthermore, as can be seen from Table 16, these differences are traceable to the forms of nitrogen in the water-insoluble portion, which is made up mostly of protein. It seems, therefore, that the water-soluble nitrogenous constituents of the leaves are the ones whose variations show consistent correlations with the occurrence of mildew.

The results of the nitrogen distribution, as a whole, lead to the following conclusions:

1. The main difference between resistant and susceptible strains lies in the water-soluble nitrogen, the susceptible strains having a considerably higher value than the resistant.
2. The forms of combination that are responsible for the difference are the free amino acid and the nonbasic nitrogen, the susceptible plants being characterized by having higher values than the resistant plants.
3. The total nitrogen of the resistant strains has a higher proportion of protein than that of the susceptible ones, the difference being due to the protein nitrogen of nonbasic character.
4. Strains which have the same genetic composition with respect to their reactions to mildew have also practically the same nitrogen distribution.
5. In pairs of reciprocal hybrids both of which are susceptible, the water-soluble nitrogen and the free amino acid are comparatively high and there is not much difference in the distribution.
6. The parent species exhibit the same characteristic differences as the contrasting reciprocal hybrids derived from them.

### THE CARBOHYDRATES

The significant differences in the sugar content of the *Oenothera* strains are shown in Table 17. Other carbohydrates do not show consistent correlations with mildew resistance. When the plants are approaching maturity and the mildew on the leaves of the susceptible plants is no longer growing, the amounts of sugar in the resistant and in the susceptible strains do not differ considerably. Furthermore, the slight variation observed is not always in the same direction. In the majority of cases, the sugar content of the resistant strains is higher than that of the susceptible ones. This observation is based upon the analyses of the samples collected in 1920. However,

when the same strains were grown in the following years and their leaves collected at the time when the plants were in active growth, at which time the mildew was also most active, the results of the sugar determinations were strikingly correlated with infection.

The figures for 1921 and 1922 in Table 17 show that the total sugar by acid hydrolysis and the free reducing sugar are higher in the resistant strains than in the susceptible ones. Moreover, in the pair in which both reciprocal hybrids are susceptible (key Nos. 17b–18b), the amount of sugar is extremely low, whereas senescent leaves of the same pair had a high sugar content in 1920. It was thought at first that this variation might be ascribed to the fact that in the susceptible strains the mildew is continually using a part of the sugar. It also seemed possible that in the leaves covered with mildew photosynthesis would be retarded. If these factors operated, one would expect to find more sugar in the full-grown leaves before infection than after. With this point in mind, the sugar content was determined in the leaves of different strains of *Oenothera* collected at two different periods of growth. The results in Table 18 are not in accord with the expectation, for it can be seen that in the case of the resistant strains the sugar content for the two periods is comparatively high, while in the susceptible strains the amount of sugar is even less in the leaves collected before infection than in those collected after infection. When the leaves of the resistant strains are compared with those of the susceptible ones of the same age, the resistant strains are characterized by higher sugar content than the susceptible ones. The results obtained suggest that the difference in sugar content must be due to the constitutional peculiarities of the resistant and the susceptible plants.

Still another possibility to be considered is the rate of translocation of the sugar from the leaves to other parts of the plants, for it might be that the movement of materials proceeds at a greater rate in the vegetatively more vigorous susceptible plants than in the resistant plants. If this supposition were correct, one would have to view the low sugar of the luxuriant susceptible plants, in contrast to the high sugar of the smaller, less rapidly growing resistant plants, as due to the removal of sugar to growing points and to ripening fruits. After all parts of the plant had come to maturity, the differ-

TABLE 17.—*Determination of carbohydrates in the leaves of different strains of Oenothera.*

[Percentage of dry weight.]

| Key No. | Name of strain. | Characteristic of strain. | Year of collection. | Total sugar by acid hydrolysis. | Free reducing sugar. | Reducing sugar freed by hydrolysis. | Pentosans. | Starch. |
|---|---|---|---|---|---|---|---|---|
| 1 | Resistant *Oe. pratincola* (a segregate from species hybrid) | Resistant | 1920 | 8.34 | 6.14 | 2.20 | 7.21 | 4.65 |
| 1a | | | 1921 | 9.65 | 8.62 | 1.03 | 7.59 | 5.10 |
| 1b | | | 1922 | 13.82 | 12.73 | 1.09 | | |
| 2 | Mildewed *Oe. pratincola* (a segregate from species hybrid) | Susceptible | 1920 | 8.17 | 6.66 | 1.51 | 6.39 | 4.37 |
| 2a | | | 1921 | 5.18 | 4.55 | 0.63 | 6.99 | 4.80 |
| 2b | | | 1922 | 8.79 | 8.43 | 0.36 | | |
| 3 | *Oe. pratincola* hyb. *immunis* | Resistant | 1920 | 12.68 | 7.44 | 5.24 | 7.80 | 5.03 |
| 3a | | | 1921 | 6.02 | 5.05 | 0.97 | 7.84 | 5.68 |
| 3b | | | 1922 | 10.62 | 10.23 | 0.39 | | |
| 4 | Mildewed *Oe. pratincola* (a segregate from species hybrid) | Susceptible | 1920 | 9.42 | 6.93 | 2.49 | 6.55 | 4.87 |
| 4a | | | 1921 | 2.79 | 2.17 | 0.62 | 7.40 | 4.32 |
| 4b | | | 1922 | 7.58 | 7.20 | 0.38 | | |
| 5 | *Oe. mississippiensis* × *Oe. pratincola* hyb. *immunis* | Resistant | 1920 | 7.81 | 6.76 | 1.05 | 7.45 | 4.95 |
| 5a | | | 1921 | 4.73 | 4.23 | 0.50 | 8.10 | 3.95 |
| 6 | *Oe. pratincola* hyb. *immunis* × *Oe. mississippiensis* | Susceptible | 1920 | 8.28 | 6.97 | 1.31 | 6.50 | 4.78 |
| 6a | | | 1921 | 4.34 | 4.18 | 0.16 | 7.00 | 3.48 |
| 8 | *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis* | Resistant | 1921 | 7.33 | 6.93 | 0.40 | 8.14 | 5.23 |
| 8a | | | 1922 | 9.85 | 8.41 | 1.44 | | |
| 9 | *Oe. pratincola* hyb. *immunis* × *Oe. cinerescens* | Susceptible | 1921 | 6.85 | 6.53 | 0.31 | 6.63 | 5.10 |
| 9a | | | 1922 | 6.40 | 5.79 | 0.61 | | |
| 17 | *Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *immunis* | do | 1920 | 10.37 | 7.44 | 2.93 | 6.80 | 4.32 |
| 17b | | | 1922 | 2.66 | 1.82 | 0.84 | | |
| 18 | *Oe. pratincola* hyb. *immunis* × *Oe. "biennis Chicago"* | do | 1920 | 10.72 | 8.75 | 1.97 | 6.88 | 4.57 |
| 18b | | | 1922 | 2.30 | 1.97 | 0.33 | | |
| 29a | *Oe. pratincola* hyb. *immunis* | Resistant | 1922 | 9.90 | 9.58 | 0.42 | 8.36 | |
| 34 | *Oe. pratincola* hyb. *rubricalyx* | Susceptible | 1922 | 8.27 | 7.62 | 0.65 | 8.24 | |
| 35 | *Oe. "biennis Chicago"* | do | 1922 | 6.25 | 5.34 | 0.91 | 8.06 | |

TABLE 18.—*Determination of sugar in the leaves of different strains of Oenothera collected at two different periods of growth during 1922.*

| Key No. | Name of strain. | Date of collection. | Condition of strain during collection. | Total sugar by acid hydrolysis. | Free reducing sugar. | Reducing sugar freed by hydrolysis. |
|---|---|---|---|---|---|---|
| | | | | *Per cent.* | *Per cent.* | *Per cent.* |
| 1b | Resistant *Oe. pratincola* (a segregate from species hybrid). | July 19 | No mildew | 11.37 | 10.51 | 0.86 |
| | | August 30 | do | 13.82 | 12.73 | 1.09 |
| 2b | Mildewed *Oe. pratincola* (a segregate from species hybrid). | July 19 | do | 8.79 | 8.43 | 0.36 |
| | | August 30 | Mildew | 10.56 | 9.37 | 1.19 |
| 3b | *Oe. pratincola* hyb. *immunis* | July 5 | No mildew | 10.62 | 10.23 | 0.39 |
| | | August 31 | do | 10.64 | 10.04 | 0.60 |
| 4b | Mildewed *Oe. pratincola* (a segregate from species hybrid). | July 5 | do | 7.58 | 7.20 | 0.38 |
| | | August 31 | Mildew | 7.58 | 6.75 | 0.83 |
| 8a | *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis* | July 5 | No mildew | 11.20 | 10.63 | 0.57 |
| | | August 30 | do | 9.85 | 8.41 | 1.44 |
| 9a | *Oe. pratincola* hyb. *immunis* × *Oe. cinerescens* | July 5 | do | 3.71 | 2.99 | 0.72 |
| | | August 30 | Mildew | 6.40 | 5.79 | 0.61 |
| 29a | *Oe. pratincola* hyb. *immunis* | July 6 | No mildew | 17.32 | 16.66 | 0.66 |
| | | August 28 | do | 9.90 | 9.58 | 0.42 |
| 34 | *Oe. pratincola* hyb. *rubricalyx* | July 5 | do | 8.16 | 7.56 | 0.60 |
| | | August 28 | Mildew | 8.27 | 7.62 | 0.65 |
| 35 | *Oe. "biennis Chicago"* | July 5 | No mildew | 8.10 | 7.70 | 0.40 |
| | | August 28 | Mildew | 6.25 | 5.34 | 0.91 |

ence should cease to exist. The results are not inconsistent with this hypothesis which, to be proved, would require elaborate experimentation. In any event, it would remain true that the low sugar of the susceptible strains is a constitutional peculiarity, and not due to modification by the mildew.

With respect to the pentosan in the leaves, Table 17 seems to show that the resistant strains contain more than the susceptible ones. However, when the determination was extended to other strains (see Table 19), it was found that pairs of reciprocal hybrids in which both members are susceptible may also show a high pentosan content. Consequently, the results do not permit any possible interpretation. One significant feature of the pentosan determination, however, is that strains similar in genetic composition also have nearly the same percentage of pentosan. This can be noticed in crosses between *Oe. mississippiensis* and *Oe. cinerescens* where one of the hybrids is a metaclinic (key Nos. 13a and 14).

The amount of starch in the leaves of the resistant and the susceptible strains does not differ considerably.

TABLE 19.—*Determination of pentosans in the leaves of different strains of Oenothera.*

| Key No. | Name of strain. | Characteristic of strain. | Pentosans in 1920. | Pentosans in 1921. |
|---|---|---|---|---|
| | | | *Per cent.* | *Per cent.* |
| 1 | Resistant *Oe. pratincola* (a segregate from species hybrid). | Resistant | 7.21 | |
| 1a | | | | 7.59 |
| 2 | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 6.39 | |
| 2a | | | | 6.99 |
| 3 | *Oe. pratincola* hyb. *immunis* | Resistant | 7.80 | |
| 3a | | | | 7.84 |
| 4 | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 6.55 | |
| 4a | | | | 7.40 |
| 5 | *Oe. mississippiensis* × *Oe. pratincola* hyb. *immunis.* | Resistant | 7.45 | |
| 5a | | | | 8.10 |
| 6 | *Oe. pratincola* hyb. *immunis* × *Oe. mississippiensis.* | Susceptible | 6.50 | |
| 6a | | | | 7.00 |
| 8 | *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis.* | Resistant | | 8.14 |
| 9 | *Oe. pratincola* hyb. *immunis* × *Oe. cinerescens.* | Susceptible | | 6.63 |
| 10 | *Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *immunis.* | Resistant | 7.56 | |
| 11 | *Oe. pratincola* hyb. *immunis* × *Oe. "biennis Chicago."* | Susceptible | 6.91 | |
| 13a | *Oe. mississippiensis* × *Oe. cinerescens* | do | | 7.97 |
| 14 | *Oe. cinerescens* × *Oe. mississippiensis*, metaclinic hybrid. | do | | 8.29 |
| 17 | *Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx.* | do | 6.80 | |
| 17a | | | | 7.30 |
| 18 | *Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago."* | do | 6.88 | |
| 18a | | | | 7.24 |
| 19a | *Oe. mississippiensis* × *Oe. "biennis Chicago."* | do | | 6.80 |
| 21a | *Oe. "biennis Chicago" Oe. mississippiensis* | do | | 7.61 |
| 22a | *Oe. mississippiensis* × *Oe. pratincola* hyb. *rubricalyx.* | do | | 8.11 |
| 23a | *Oe. pratincola* hyb. *rubricalyx* × *Oe. mississippiensis.* | do | | 6.32 |
| 24 | *Oe. pratincola* mut. *nitidissima* | Resistant | | 7.82 |
| 24a | do | do | | 7.90 |
| 25 | do | Susceptible | | 7.22 |
| 25a | do | do | | 7.13 |
| 29a | *Oe. pratincola* hyb. *immunis* | Resistant | | 8.36 |
| 34 | *Oe. pratincola* hyb. *rubricalyx* | Susceptible | | 8.24 |
| 35 | *Oe. "biennis Chicago"* | do | | 8.06 |

### THE TANNIN

In the determination of the sugar, it was noticed that the amount of Fehling's solution reduced by the unclarified extract of the leaves was much higher than that for the same extract

after it was clarified with lead subacetate solution. Another striking thing was that the unclarified extract of the resistant strains showed higher reduction than that of the susceptible ones. Table 20 shows the results alluded to. It was obvious that there must be in the extract some substance other than sugar that possessed the power of reducing Fehling's solution and that differed from sugar in being precipitated by the lead subacetate. It could not be a glucoside of the usual type, for the high reduction of Fehling's solution was noticed in the extract before hydrolysis. Moreover, the percentage of reducing sugar freed by hydrolysis does not differ very much in the clarified and the unclarified extracts. The extract when tested with ferric chloride solution gave a deep blackish blue color, suggesting that tannin must be the other substance in the extract which reduced the Fehling's solution and was precipitated with lead subacetate. Accordingly, a tannin determination was carried out for a number of strains and the results in Table 21 are in accordance with the expectation. Some characteristic features that are shown in the table are:

1. In pairs of reciprocal hybrids, the resistant one contains a much higher percentage of tannin than the susceptible one, the difference varying from 50 to 150 per cent.

2. Those elementary species which are resistant have higher tannin content than those which are susceptible, the only exception being *Oe. "biennis Chicago,"* a susceptible species in which the tannin is rather high.

3. Of pairs in which both reciprocal hybrids are susceptible, the tannin content is correspondingly low.

4. The metaclinic hybrid *Oe. cinerescens* × *Oe. mississippiensis* and its reciprocal are found to contain almost the same amount of tannin.

5. Of the two strains of *Oe. pratincola* mut. *nitidissima* the resistant one is higher in tannin than the susceptible one.

### THE CRUDE FIBER

The determinations of crude fiber that are given in Table 22 indicate that the resistant strains have higher crude fiber than the susceptible ones.

### THE WATER-SOLUBLE ACIDS

There is a much greater amount of water-soluble acid in the leaves of the resistant hybrids than in the leaves of those which are susceptible (see Table 23). In the elementary species, the resistant ones are also conspicuously higher in total acid.

TABLE 20.—*Reducing sugar in the leaves of the different strains of Oenothera.*

[Percentage of dry weight.]

| Key No. | Name of strain. | Characteristic of strain. | Year of collection. | Clarified solution. | | | Unclarified solution. | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Reducing sugar before inversion. | Reducing sugar after inversion. | Reducing sugar freed by hydrolysis. | Reducing sugar before inversion. | Reducing sugar after inversion. | Reducing sugar freed by hydrolysis. |
| 1 | Resistant *Oe. pratincola* (a segregate from species hybrid). | Resistant | 1920 | 6.14 | 8.34 | 2.20 | 18.27 | 20.63 | 2.36 |
| 1a | | | 1921 | 8.62 | 9.65 | 1.03 | 21.70 | 22.64 | 0.94 |
| 2 | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 1920 | 6.66 | 8.17 | 1.51 | 13.60 | 15.95 | 2.35 |
| 2a | | | 1921 | 4.55 | 5.18 | 0.63 | 12.22 | 13.56 | 1.34 |
| 3 | *Oe. pratincola* hyb. *immunis* | Resistant | 1920 | 7.44 | 12.68 | 5.24 | 18.05 | 23.07 | 5.02 |
| 3a | | | 1921 | 5.05 | 6.02 | 0.97 | 18.63 | 20.81 | 2.18 |
| 4 | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 1920 | 6.93 | 9.42 | 2.49 | 15.69 | 20.18 | 4.49 |
| 4a | | | 1921 | 2.17 | 2.79 | 0.62 | 10.27 | 11.29 | 1.02 |
| 5 | *Oe. mississippiensis* × *Oe. pratincola* hyb. *immunis*. | Resistant | 1920 | 6.76 | 7.81 | 1.05 | 16.41 | 18.84 | 2.43 |
| 6 | *Oe. pratincola* hyb. *immunis* × *Oe. mississippiensis*. | Susceptible | 1920 | 6.97 | 8.28 | 1.31 | 13.47 | 15.49 | 2.07 |
| 8 | *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis* | Resistant | 1921 | 6.93 | 7.33 | 0.40 | 15.03 | 16.87 | 1.84 |
| 9 | *Oe. pratincola* hyb. *immunis* × *Oe. cinerescens* | Susceptible | 1921 | 6.53 | 6.85 | 0.31 | 11.46 | 12.90 | 1.44 |
| 17 | *Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx*. | ----do | 1920 | 7.44 | 10.37 | 2.93 | 21.24 | 24.04 | 2.80 |
| 18 | *Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago"*. | ----do | 1920 | 8.75 | 10.72 | 1.97 | 18.46 | 20.35 | 1.89 |

In the case of the strains that have the same zygotic composition as far as their reaction with the mildew is concerned, the percentage of the water-soluble acid does not differ to a great extent. This is shown by the metaclinic hybrid *Oe. cinerescens* × *Oe. mississippiensis* and its reciprocal. The parent species exhibit the same characteristic as their corresponding hybrids. For example, the water-soluble content in the resistant *Oe. pratincola* hyb. *immunis* is almost twice as high as in *Oe. pratincola* hyb. *rubricalyx*, a susceptible parent.

TABLE 21.—*Percentage of tannin in the leaves of different strains of Oenothera.*

[Expressed in terms of gallotanic acid.]

| Key No. | Name of strain. | Characteristic of strain. | Tannin in 1920. | Tannin in 1921. | Tannin in 1922. |
|---|---|---|---|---|---|
| | | | *Per cent.* | *Per cent.* | *Per cent.* |
| 1 | Resistant *Oe. pratincola* (a segregate from species hybrid). | Resistant | 13.24 | | |
| 1a | | | | 10.08 | |
| 1b | | | | | 9.87 |
| 2 | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 5.06 | | |
| 2a | | | | 3.47 | |
| 2b | | | | | 5.05 |
| 3 | *Oe. pratincola* hyb. *immunis* | Resistant | 8.96 | | |
| 3a | | | | 9.79 | |
| 3b | | | | | 10.22 |
| 4 | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 5.41 | | |
| 4a | | | | 5.43 | |
| 4b | | | | | 6.22 |
| 5 | *Oe. mississippiensis* × *Oe. pratincola* hyb. *immunis*. | Resistant | 6.87 | | |
| 5a | | | | 6.58 | |
| 6 | *Oe. pratincola* hyb. *immunis* × *Oe. mississippiensis*. | Susceptible | 3.83 | | |
| 6a | | | | 4.49 | |
| 8 | *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*. | Resistant | | 5.49 | |
| 8a | | | | | 8.30 |
| 9 | *Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*. | Susceptible | | 2.22 | |
| 9a | | | | | 3.58 |
| 10 | *Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *immunis*. | Resistant | 9.27 | | |
| 11 | *Oe. pratincola* hyb. *immunis* × *Oe. "biennis Chicago."* | Susceptible | 4.41 | | |
| 13a | *Oe. mississippiensis* × *Oe. cinerescens* | do | | 3.94 | |
| 13b | | | | | 5.83 |
| 14 | *Oe. cinerescens* × *Oe. mississippiensis*, metaclinic hybrid. | do | | 3.54 | |
| 14a | | | | | 5.19 |
| 17 | *Oe. "biennis Chicago"* × *Oe. pranticola* hyb. *rubricalyx*. | do | 9.14 | | |
| 17a | | | | 6.82 | |
| 17b | | | | | 9.42 |
| 18 | *Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago."* | do | 6.30 | | |
| 18a | | | | 8.52 | |
| 18b | | | | | 9.84 |
| 22 | *Oe. mississippiensis* × *Oe. pratincola* hyb. *rubricalyx*. | do | 6.09 | | |
| 22a | | | | 4.99 | |

TABLE 21.—*Percentage of tannin in the leaves of different strains of Oenothera*—Continued.

| Key No. | Name of strain. | Characteristic of strain. | Tannin in 1920. | Tannin in 1921. | Tannin in 1922. |
|---|---|---|---|---|---|
| | | | *Per cent.* | *Per cent.* | *Per cent.* |
| 23 | *Oe. pratincola* hyb. *rubricalyx* × *Oe. missis-* | Susceptible | 6.77 | | |
| 23a | *sippiensis.* | | | 5.35 | |
| 24 | *Oe. pratincola* mut. *nitidissima* | Resistant | | 10.96 | |
| 24a | do | do | | 11.12 | |
| 25 | do | Susceptible | | 8.66 | |
| 26 | *Oe. numismatica* | Resistant | | 12.74 | |
| 27 | *Oe. pratincola* mut. *simulans* | do | | 10.05 | |
| 28 | *Oe. pratincola* mut. *simulans rubricalyx* | Susceptible | | 8.68 | |
| 29 | *Oe. pratincola* hyb. *immunis* | Resistant | | 9.95 | |
| 29a | | | | | 10.48 |
| 30 | *Oe. reynoldsii* × *Oe. pratincola* | do | | 8.82 | |
| 31 | *Oe. pratincola* (susceptible) | Susceptible | | 6.19 | |
| 32 | *Oe. mississippiensis* | do | | 6.99 | |
| 33 | *Oe. reynoldsii* | Resistant | | 11.03 | |
| 34 | *Oe. pratincola* hyb. *rubricalyx* | Susceptible | | | 5.10 |
| 35 | *Oe.* "*biennis Chicago*" | | | | 10.85 |

TABLE 22.—*Determination of crude fiber in the leaves of different strains of Oenothera.*

| Key No. | Name of strain. | Characteristic of strain. | Crude fiber in 1920. | Crude fiber in 1921. |
|---|---|---|---|---|
| | | | *Per cent.* | *Per cent.* |
| 1 | Resistant *Oe. pratincola* (a segregate from | Resistant | 7.77 | |
| 1a | species hybrid). | | | 6.02 |
| 2 | Mildewed *Oe. pratincola* (a segregate from | Susceptible | 5.60 | |
| 2a | species hybrid). | | | 5.85 |
| 3 | *Oe. pratincola* hyb. *immunis* | Resistant | 6.41 | |
| 3a | | | | 6.49 |
| 4 | Mildewed *Oe. pratincola* (a segregate from | Susceptible | 5.43 | |
| 4a | species hybrid). | | | 6.15 |
| 5 | *Oe. mississippiensis* × *Oe. pratincola* hyb. | Resistant | 7.26 | |
| 5a | *immunis.* | | | 7.45 |
| 6 | *Oe. pratincola* hyb. *immunis* × *Oe. mississip-* | Susceptible | 6.39 | |
| 6a | *piensis.* | | | 6.80 |
| 8 | *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis* | Resistant | | 7.29 |
| 9 | *Oe. pratincola* hyb. *immunis* × *Oe. cinerescens* | Susceptible | | 6.19 |
| 24 | *Oe. pratincola* mut. *nitidissima* | Resistant | | 7.12 |
| 24a | do | do | | 7.33 |
| 25 | do | Susceptible | | 6.53 |
| 25a | do | do | | 6.41 |

THE TOTAL ASH

The results of the total ash determination in Table 24 indicate that, with a few exceptions, the susceptible strains contain much

more total ash than the resistant ones. Exceptions were noted in hybrids resulting from *Oe. mississippiensis* and *Oe. pratincola* hyb. *immunis*, *Oe. "biennis Chicago"* and *Oe. pratincola* hyb. *immunis*, and *Oe. cinerescens* and *Oe. mississippiensis*. However, in these exceptional cases the percentage of total ash in the resistant hybrids is only slightly higher than in their susceptible reciprocals.

TABLE 23.—*Determination of the water-soluble acid in the leaves of different strains of Oenothera.*

[Expressed in milligrams of sodium hydroxide required to neutralize the acid from 1 gram of moisture-free leaves.]

| Key No. | Name of strain. | Characteristic of strain. | 1920 | 1921 | 1922 |
|---|---|---|---|---|---|
| 1 | Resistant *Oe. pratincola* (a segregate from species hybrid). | Resistant | 37.56 | | |
| 1a | | | | 25.21 | |
| 1b | | | | | 21.61 |
| 2 | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 25.36 | | |
| 2a | | | | 20.65 | |
| 2b | | | | | 17.24 |
| 3 | *Oe. pratincola* hyb. *immunis* | Resistant | 24.16 | | |
| 3a | | | | 23.28 | |
| 3b | | | | | 25.60 |
| 4 | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 23.12 | | |
| 4a | | | | 21.92 | |
| 4b | | | | | 19.28 |
| 5 | *Oe. mississippiensis* × *Oe. pratincola* hyb. *immunis*. | Resistant | 30.04 | | |
| 5a | | | | 20.02 | |
| 6 | *Oe. pratincola* hyb. *immunis* × *Oe. mississippiensis*. | Susceptible | 24.16 | | |
| 6a | | | | 21.96 | |
| 8 | *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*. | Resistant | | 21.36 | |
| 8a | | | | | 19.52 |
| 9 | *Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*. | Susceptible | | 13.72 | |
| 9a | | | | | 13.68 |
| 10 | *Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *immunis*. | Resistant | 32.04 | | |
| 11 | *Oe. pratincola* hyb. *immunis* × *Oe. "biennis Chicago."* | Susceptible | 25.24 | | |
| 12 | *Oe. cinerescens* × *Oe. mississippiensis* | Resistant | 24.64 | | |
| 13 | *Oe. mississippiensis* × *Oe. cinerescens* | Susceptible | 23.84 | | |
| 13a | | | | 14.72 | |
| 13b | | | | | 18.64 |
| 14 | *Oe. cinerescens* × *Oe. mississippiensis*, metaclinic hybrid. | do | | 13.44 | |
| 14a | | | | | 18.26 |
| 24 | *Oe. pratincola* mut. *nitidissima* | Resistant | | 30.40 | |
| 24a | do | do | | 31.64 | |
| 25 | do | Susceptible | | 26.72 | |
| 25a | do | do | | 27.15 | |
| 29a | *Oe. pratincola* hyb. *immunis* | Resistant | | | 23.85 |
| 34 | *Oe. pratincola* hyb. *rubricalyx* | Susceptible | | | 12.43 |
| 35 | *Oe. "biennis Chicago"* | do | | | 19.15 |

### THE INORGANIC CONSTITUENTS OF THE ASH

In order to find out the constituents of the ash that differ to a marked degree in the resistant and the susceptible strains, partial ash analyses were made. The results, given in Table 25, reveal that the main difference is found in the calcium and sulphur content. The percentages of these two elements are higher in the leaves of the susceptible strains than in those of the resistant ones. To a small degree the susceptible strains also contain more phosphorus and potassium. When the different constituents are expressed as percentages of the total ash (Table 26), the results for potassium and phosphorus are not concordant, while those for calcium and sulphur are; that is, the total ash of the susceptible strains contains more calcium and sulphur than does that of the resistant ones.

In view of the marked differences noted in the percentage of total nitrogen, total ash, sugar, tannin, and water-soluble acid in the resistant and the susceptible strains, further analyses were made to see if these differences existed in the plants before the susceptible strains were mildewed. These five constituents were, therefore, determined in the leaves of several strains collected at two different periods of growth; one when the susceptible strains were not yet infected, and the other when they were infected. The results in Table 27 show some interesting facts. In the first place, the characteristic chemical differences between the resistant and the susceptible strains are found to be exactly the same, both before and after infection. In fact, the differences are even more pronounced before infection, for the resistant strains are exceedingly high in tannin and water-soluble acid, while the susceptible ones contain a great amount of total ash and total nitrogen. Another point of interest is that the amounts of the total nitrogen, tannin, total ash, and water-soluble acid do not differ much for the two periods in the resistant strains; whereas, in the susceptible, the percentage of these four constituents varies considerably for the two periods, the variation being in such direction as to render their difference from the resistant ones far more significant. The results obtained for the sugar have already been presented separately in Table 18, in connection with the carbohydrates.

### EXTENSION OF THE WORK TO OTHER PLANTS

To determine whether the chemical differences existing in the different strains of *Oenothera* are also found in other

TABLE 24.—*Percentage of total ash in the leaves of different strains of Oenothera.*

| Key No. | Name of strain. | Characteristic of strain. | Total ash in 1920. | Total ash in 1921. | Total ash in 1922. |
|---|---|---|---|---|---|
| | | | *Per cent.* | *Per cent.* | *Per cent.* |
| 1 | Resistant *Oe. pratincola* (a segregate from species hybrid). | Resistant | 11.09 | | |
| 1a | | | | 9.04 | |
| 1b | | | | | 8.77 |
| 2 | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 13.40 | | |
| 2a | | | | 13.14 | |
| 2b | | | | | 11.16 |
| 3 | *Oe. pratincola* hyb. *immunis* | Resistant | 11.29 | | |
| 3a | | | | 9.57 | |
| 3b | | | | | 10.81 |
| 4 | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 13.10 | | |
| 4a | | | | 13.28 | |
| 4b | | | | | 13.93 |
| 5 | *Oe. mississippiensis* × *Oe. pratincola* hyb. *immunis*. | Resistant | 11.27 | | |
| 5a | | | | 12.81 | |
| 6 | *Oe. pratincola* hyb. *immunis* × *Oe. mississippiensis*. | Susceptible | 10.16 | | |
| 6a | | | | 12.06 | |
| 8 | *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*. | Resistant | | 10.65 | |
| 8a | | | | | 11.58 |
| 9 | *Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*. | Susceptible | | 13.54 | |
| 9a | | | | | 15.03 |
| 10 | *Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *immunis*. | Resistant | 11.90 | | |
| 11 | *Oe. pratincola* hyb. *immunis* × *Oe. "biennis Chicago."* | Susceptible | 11.49 | | |
| 12 | *Oe. cinerescens* × *Oe. mississippiensis* | Resistant | 13.63 | | |
| 13 | *Oe. mississippiensis* × *Oe. cinerescens* | Susceptible | 13.49 | | |
| 13a | | | | 13.66 | |
| 13b | | | | | 14.83 |
| 14 | *Oe. cinerescens* × *Oe. mississippiensis*, metaclinic hybrid. | do | | 14.00 | |
| 14b | | | | | 14.68 |
| 15 | *Oe. pratincola* × *Oe. reynoldsii* (*Oe. pratincola* hyb. *amycosa*). | Resistant | 10.84 | | |
| 16 | *Oe. reynoldsii* × *Oe. pratincola* | Susceptible | 11.26 | | |
| 17a | *Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx*. | do | | 11.18 | |
| 17b | | | | | 11.08 |
| 18 | *Oe. pratincola* hyb. *rubricalyx* × *Oe. "biennis Chicago"*. | do | | 11.73 | |
| 18a | | | | | 12.96 |
| 24 | *Oe. pratincola* mut. *nitidissima* | Resistant | | 10.44 | |
| 24a | do | do | | 9.97 | |
| 25 | do | Susceptible | | 11.88 | |
| 25a | do | do | | 11.10 | |
| 26 | *Oe. numismatica* | Resistant | | 9.76 | |
| 27 | *Oe. pratincola* mut. *simulans* | do | | 10.63 | |
| 28 | *Oe. pratincola* mut. *simulans rubricalyx* | | | 11.39 | |
| 29a | *Oe. pratincola* hyb. *immunis* | Resistant | | | 7.47 |
| 31 | *Oe. pratincola* | Susceptible | | 12.94 | |
| 32 | *Oe. mississippiensis* | do | | 11.51 | |
| 33 | *Oe. reynoldsii* | Resistant | | 10.60 | |
| 34 | *Oe. pratincola* hyb. *rubricalyx* | Susceptible | | | 13.04 |
| 35 | *Oe. "biennis Chicago"* | do | | | 11.55 |

TABLE 25.—*Constituents of the ash in the leaves of different strains of Oenothera.*

| Key No. | Name of strain. | Characteristic of strain. | Total ash. | Constituents of ash, expressed as percentage of moisture-free leaves. | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $SiO_2$ | CaO | MgO | $K_2O$ | $Na_2O$ | $P_2O_5$ | $SO_3$ |
| 1b | Resistant *Oe. pratincola* (a segregate from species hybrid). | Resistant | 8.77 | 0.346 | 3.32 | 1.04 | 0.713 | 0.139 | 0.425 | 0.541 |
| 2b | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 11.16 | 0.414 | 4.51 | 1.10 | 1.07 | 0.134 | 0.628 | 0.875 |
| 3b | *Oe. pratincola* hyb. *immunis* | Resistant | 10.81 | 0.445 | 4.24 | 1.07 | 1.08 | 0.088 | 0.560 | 0.763 |
| 4b | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 13.93 | 0.549 | 5.87 | 1.36 | 11.36 | 0.048 | 0.663 | 1.067 |
| 8a | *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*. | Resistant | 11.58 | 0.325 | 4.47 | 1.25 | 1.21 | 0.086 | 0.558 | 0.867 |
| 9a | *Oe. pratincola* hyb. *immunis* × *Oe. cinerescens*. | Susceptible | 15.03 | 0.387 | 6.80 | 1.39 | 1.41 | 0.052 | 0.688 | 1.99 |
| 29a | *Oe. pratincola* hyb. *immunis* | Resistant | 7.47 | 0.217 | 2.66 | 0.677 | 0.702 | 0.044 | 0.391 | 0.508 |
| 34 | *Oe. pratincola* hyb. *rubricalyx* | Susceptible | 13.04 | 0.539 | 5.01 | 1.48 | 1.64 | 0.177 | 0.633 | 1.17 |

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TABLE 26.—*Constituents of the ash in the leaves of different strains of Oenothera.*

| Key No. | Name of strain. | Characteristic of strain. | Expressed as percentage of total ash. | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | $SiO_2$ | CaO | MgO | $K_2O$ | $Na_2O$ | $P_2O_5$ | $SO_3$ |
| 1b | Resistant *Oe. pratincola* (a segregate from species hybrid). | Resistant | 3.94 | 37.81 | 11.59 | 8.13 | 1.59 | 4.85 | 6.18 |
| 2b | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 3.71 | 40.45 | 9.89 | 9.61 | 1.20 | 5.63 | 7.85 |
| 3b | *Oe. pratincola* hyb. *immunis* | Resistant | 4.12 | 39.26 | 10.35 | 10.02 | 0.812 | 5.18 | 7.06 |
| 4b | Mildewed *Oe. pratincola* (a segregate from species hybrid). | Susceptible | 3.94 | 42.15 | 9.78 | 9.74 | 0.342 | 4.76 | 7.65 |
| 8a | *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis* | Resistant | 2.81 | 38.61 | 10.76 | 10.46 | 0.745 | 4.82 | 7.48 |
| 9a | *Oe. pratincola* hyb. *immunis* × *Oe. cinerescens* | Susceptible | 2.58 | 45.25 | 9.24 | 9.37 | 0.348 | 4.58 | 13.23 |
| 29a | *Oe. pratincola* hyb. *immunis* | Resistant | 2.90 | 35.61 | 9.07 | 9.40 | 0.590 | 5.24 | 6.71 |
| 34 | *Oe. pratincola* hyb. *rubricalyx* | Susceptible | 4.13 | 38.41 | 11.32 | 12.54 | 1.36 | 4.86 | 8.96 |

TABLE 27.—*Total nitrogen, tannin, total ash, and water-soluble acid in the leaves of different strains of Oenothera collected at two different periods of growth during 1922.*

| Key No. | Name of strain. | Date of collection. | Condition of strain during collection. | Total nitrogen. | Tannins. | Total ash. | Water-soluble acid in terms of mgm. NaOH.[a] |
|---|---|---|---|---|---|---|---|
| | | | | *Per cent.* | *Per cent.* | *Per cent.* | |
| 1b | Resistant *Oe. pratincola* (a segregate from species hybrid) | July 19 | No mildew | 2.40 | 10.80 | 8.21 | 30.32 |
| | | August 30 | do | 2.54 | 9.87 | 8.77 | 21.61 |
| 2b | Mildewed *Oe. pratincola* (a segregate from species hybrid) | July 19 | do | 3.63 | 4.30 | 12.70 | 21.28 |
| | | August 30 | Mildew | 3.03 | 5.05 | 11.16 | 17.24 |
| 3b | *Oe. pratincola* hyb. *immunis* | July 6 | No mildew | 2.06 | 10.85 | 7.54 | 26.36 |
| | | August 30 | do | 2.29 | 10.22 | 10.81 | 25.60 |
| 4b | Mildewed *Oe. pratincola* (a segregate from species hybrid) | July 6 | do | 3.86 | 3.98 | 14.19 | 17.24 |
| | | August 30 | Mildew | 3.08 | 6.22 | 13.93 | 19.28 |
| 8a | *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis* | July 6 | No mildew | 2.54 | 9.30 | 10.19 | 26.48 |
| | | August 30 | do | 2.45 | 8.30 | 11.58 | 19.52 |
| 9a | *Oe. pratincola* hyb. *immunis* × *Oe. cinerescens* | July 6 | do | 3.94 | 2.38 | 16.57 | 17.76 |
| | | August 30 | Mildew | 2.81 | 3.58 | 15.03 | 13.68 |
| 13b | *Oe. mississippiensis* × *Oe. cinerescens* | July 6 | No mildew | 3.81 | 5.56 | 15.12 | 20.88 |
| | | August 30 | Mildew | 2.69 | 5.83 | 14.83 | 18.64 |
| 14a | *Oe. cinerescens* × *Oe. mississippiensis*, metaclinic hybrid | July 6 | No mildew | 3.67 | 5.71 | 14.72 | 20.80 |
| | | August 30 | Mildew | 2.64 | 6.19 | 14.68 | 18.26 |
| 29a | *Oe. pratincola* hyb. *immunis* | July 6 | No mildew | 2.31 | 10.69 | 7.75 | 25.68 |
| | | August 28 | do | 2.54 | 10.48 | 7.47 | 23.85 |
| 34 | *Oe. pratincola* hyb. *rubricalyx* | July 6 | do | 3.84 | 2.46 | 14.65 | 13.96 |
| | | August 28 | Mildew | 2.95 | 5.10 | 13.04 | 12.43 |
| 35 | *Oe. "biennis Chicago"* | July 5 | No mildew | 3.49 | 5 47 | 12.89 | 21.20 |
| | | August 28 | Mildew | 2.91 | 10.85 | 11.55 | 19.15 |

[a] The water-soluble acid is expressed as the number of milligrams of sodium hydroxide required to neutralize the acid from 1 gram of moisture-free leaves.

plants that are infected by other forms of powdery mildew, the determination for total nitrogen, total ash, tannin, and water-soluble acid was extended to *Syringa vulgaris, Desmodium canadensis, Helianthus giganteus,* and *Solidago canadensis,* all of these plants having been found to have resistant and susceptible varieties. The results of the determination are given in Table 28. Except for the total ash and water-soluble acid of the strains of *Helianthus giganteus,* the findings confirm those already found in *Oenothera.* There was little time available for this work, which was undertaken through curiosity and with the knowledge that it could not be carried far. It indicates that the results for *Oenothera* are probably of general applicability, and that any of the foregoing genera, and doubtless many others, would afford material that would be equally interesting from the standpoint of genetics and disease resistance in *Oenothera.*

TABLE 28.—*Total nitrogen, tannin, total ash, and water-soluble acid (in terms of NaOH required for neutralization of acid from 1 gram of moisture-free leaves) in the leaves of some plants which were found during the summer of 1922 to have strains resistant and susceptible to powdery mildew.*

| Name of plant. | Characteristic of plant. | Total nitrogen. | Total tannin. | Total ash. | Water-soluble acid mgm. NaOH. |
|---|---|---|---|---|---|
| | | *Per cent.* | *Per cent.* | *Per cent.* | |
| *Syringa vulgaris* | Highly resistant | 1.99 | 2.52 | 9.93 | 13.40 |
| Do | Susceptible | 2.68 | 1.40 | 12.48 | 10.08 |
| *Desmodium canadensis* | Resistant | 2.88 | 4.39 | 7.32 | 11.24 |
| Do | Susceptible | 2.95 | 2.06 | 8.51 | 10.92 |
| *Helianthus giganteus* | Resistant | 1.98 | 6.36 | 17.93 | 7.16 |
| Do | Susceptible | 2.36 | 5.98 | 11.57 | 7.12 |
| *Solidago canadensis* | Resistant | 2.43 | 0.85 | 10.62 | 11.40 |
| Do | Susceptible | 2.76 | 0.003 | 12.47 | 8.48 |

## DISCUSSION OF RESULTS

The whole body of experimental data brought forward indicates that the chemical differences between the resistant and the susceptible strains of *Oenothera* are definite hereditary characteristics, and that these chemical characteristics are correlated with the degree of resistance of the various strains to mildew. This statement holds true for the nitrogen distribution, the tannin, the water-soluble acid, and the total ash. Therefore, it is possible, if the analysis of a strain is given, to predict more or less accurately what the reaction to mildew will be. It is not possible, however, to use the percentage of any one constituent as

an unfailing index. When the results of analysis are expressed as percentages it is obvious that, if one constituent is high, other constituents must be correspondingly low. A series of ratios, in which some one constituent is taken as unity, indicates more clearly than does the percentage composition the significance of variation in single constituents. Such ratios, for example, show at a glance whether or not two strains differing in percentage composition have actually the same relative composition except with regard to one constituent. For example, if the total nitrogen content of all strains under consideration is considered as unity, the ratios of the various constituents may be summarized for comparison at a glance, as in Table 29.

In Table 29 the ratios show a correlation or at least a parallelism of only two constituents, namely, total nitrogen and total ash. There seems to be no consistent quantitative interdependence among the other constituents, except that pentosan and water-soluble acid show parallel variation. The ratios with constituents other than total nitrogen taken as unity are shown in Tables 30 to 33.

If the amount of any one constituent alone determined resistance or susceptibility, one might expect to find all the other constituents showing parallel variation such as we find in the case of total nitrogen and total ash, or perhaps nonconcordant variation in several pairs of reciprocals. The former condition might be expected in forms morphologically identical but different physiologically, as indicated by unlike resistance to infection. The latter condition would be expected in morphologically unlike strains as well as in those physiologically unlike, since of course one would expect structural dissimilarities of considerable magnitude to be reflected in some way in the chemical composition. Nothing is more obvious from an examination of the tables, however, than that every resistant strain is higher in tannin and water-soluble acid than the genetically most closely related susceptible strain which may be compared with it. Conversely, the resistant strains are correspondingly low in total nitrogen and ash. The results for sugar are not concordant.

Our results, therefore, fail to point to any single constituent as the basis for resistance, and we are justified in concluding only that, when an *Oenothera* is low in nitrogen and ash and high in tannin and acid, it is a resistant strain; if the situation is reversed, the strain is susceptible. As far as we can draw conclusions from the results, we may look upon resistance as having several or many contributory factors.

TABLE 29.—*Constitution of reciprocal hybrids and mutations of Oenothera, expressed as ratios, total nitrogen being taken as unity.*

| Key No. | Name of strain. | Characteristic of strain. | Total nitrogen. | Pentosan. | Total sugar by acid hydrolysis. | Tannin. | Water-soluble acid. | Total ash. |
|---|---|---|---|---|---|---|---|---|
| 1a | Resistant *Oe. pratincola* (a segregate from species hybrid) | Resistant | 100 | 293.05 | 372.59 | 389.19 | 973.36 | 349.03 |
| 2a | Mildewed *Oe. pratincola* (a segregate from species hybrid) | Susceptible | 100 | 193.62 | 143.49 | 96.12 | 572.00 | 363.98 |
| 3a | *Oe. pratincola* hyb. *immunis* | Resistant | 100 | 281.00 | 181.00 | 350.89 | 834.40 | 342.98 |
| 4a | Mildewed *Oe. pratincola* (a segregate from species hybrid) | Susceptible | 100 | 191.21 | 56.07 | 140.31 | 566.39 | 343.14 |
| 8 | *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis* | Resistant | 100 | 252.79 | 227.63 | 168.94 | 663.33 | 330.74 |
| 9 | *Oe. pratincola* hyb. *immunis* × *Oe. cinerescens* | Susceptible | 100 | 181.64 | 187.67 | 60.82 | 375.89 | 370.96 |
| 13a | *Oe. mississippiensis* × *Oe. cinerescens* | do | 100 | 272.01 | | 134.47 | 502.38 | 466.62 |
| 14 | *Oe. cinerescens* × *Oe. mississippiensis*, metaclinic hybrid | do | 100 | 289.86 | | 123.78 | 469.93 | 489.51 |
| 24 | *Oe. pratincola* mut. *nitidissima* | Resistant | 100 | 280.28 | | 392.83 | 1,089.59 | 374.19 |
| 25 | do | Susceptible | 100 | 225.62 | | 274.05 | 845.55 | 375.94 |

TABLE 30.—*Constitution of reciprocal hybrids and mutations of Oenothera, expressed as ratios, total ash being taken as unity.*

| Key No. | Name of strain. | Characteristic of strain. | Total ash. | Pentosan. | Total nitrogen. | Total sugar by acid hydrolysis. | Tannin. | Water-soluble acid. |
|---|---|---|---|---|---|---|---|---|
| 1a | Resistant *Oe. pratincola* (a segregate from species hybrid) | Resistant | 100 | 83.95 | 28.65 | 106.74 | 111.50 | 278.85 |
| 2a | Mildewed *Oe. pratincola* (a segregate from species hybrid) | Susceptible | 100 | 53.20 | 27.47 | 39.42 | 26.41 | 157.15 |
| 3a | *Oe. pratincola* hyb. *immunis* | Resistant | 100 | 81.92 | 29.15 | 52.77 | 102.30 | 243.25 |
| 4a | Mildewed *Oe. pratincola* (a segregate from species hybrid) | Susceptible | 100 | 55.72 | 29.14 | 16.34 | 40.89 | 165.05 |
| 8 | *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis* | Resistant | 100 | 76.43 | 30.23 | 68.82 | 51.08 | 199.98 |
| 9 | *Oe. pratincola* hyb. *immunis* × *Oe. cinerescens* | Susceptible | 100 | 48.97 | 26.96 | 50.59 | 16.40 | 101.33 |
| 13a | *Oe. mississippiensis* × *Oe. cinerescens* | do | 100 | 58.35 | 21.45 | | 28.84 | 107.76 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 14 | *Oe. cinerescens* × *Oe. mississippiensis*, metaclinic hybrid | do | 100 | 59.21 | 20.43 | | 25.29 | 95.99 |
| 24 | *Oe. pratincola* mut. *nitidissima* | Resistant | 100 | 74.90 | 26.72 | | 104.98 | 291.17 |
| 25 | do | Susceptible | 100 | 60.02 | 26.59 | | 72.90 | 224.92 |

TABLE 31.—*Constitution of reciprocal hybrids and mutations of Oenothera, expressed as ratios, pentosan being taken as unity.*

| Key No. | Name of strain. | Characteristic of strain. | Pentosan. | Total nitrogen. | Total sugar by acid hydrolysis. | Tannin. | Water-soluble acid. | Total ash. |
|---|---|---|---|---|---|---|---|---|
| 1a | Resistant *Oe. pratincola* (a segregate from species hybrid) | Resistant | 100 | 34.11 | 127.09 | 132.75 | 332.02 | 119.06 |
| 2a | Mildewed *Oe. pratincola* (a segregate from species hybrid) | Susceptible | 100 | 51.64 | 74.11 | 49.64 | 295.42 | 187.98 |
| 3a | *Oe. pratincola* hyb. *immunis* | Resistant | 100 | 35.59 | 64.41 | 127.87 | 296.94 | 122.07 |
| 4a | Mildewed *Oe. pratincola* (a segregate from species hybrid) | Susceptible | 100 | 52.30 | 29.32 | 73.38 | 296.20 | 179.45 |
| 8 | *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis* | Resistant | 100 | 39.56 | 90.05 | 66.83 | 262.41 | 130.84 |
| 9 | *Oe. pratincola* hyb. *immunis* × *Oe. cinerescens* | Susceptible | 100 | 55.05 | 103.31 | 33.48 | 206.93 | 204.21 |
| 13a | *Oe. mississippiensis* × *Oe. cinerescens* | do | 100 | 36.76 | | 49.44 | 184.69 | 171.39 |
| 14 | *Oe. cinerescens* × *Oe. mississippiensis*, metaclinic hybrid | do | 100 | 34.50 | | 42.70 | 162.11 | 168.87 |
| 24 | *Oe. pratincola* mut. *nitidissima* | Resistant | 100 | 35.68 | | 140.15 | 388.72 | 133.50 |
| 25 | do | Susceptible | 100 | 44.32 | | 121.46 | 374.75 | 166.62 |

TABLE 32.—*Constitution of reciprocal hybrids and mutations of Oenothera, expressed as ratios, tannin being taken as unity.*

| Key No. | Name of strain. | Characteristic of strain. | Tannin. | Pentosan. | Total nitrogen. | Total sugar by acid hydrolysis. | Water soluble acid. | Total ash. |
|---|---|---|---|---|---|---|---|---|
| 1a | Resistant *Oe. pratincola* (a segregate from species hybrid) | Resistant | 100 | 75.30 | 25.69 | 95.73 | 250.09 | 89.68 |
| 2a | Mildewed *Oe. pratincola* (a segregate from species hybrid) | Susceptible | 100 | 201.44 | 104.03 | 149.28 | 595.09 | 378.67 |
| 3a | *Oe. pratincola* hyb. *immunis* | Resistant | 100 | 80.08 | 28.49 | 51.58 | 237.78 | 97.75 |
| 4a | Mildewed *Oe. pratincola* (a segregate from species hybrid) | Susceptible | 100 | 136.29 | 71.27 | 39.96 | 403.68 | 244.56 |
| 8 | *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis* | Resistant | 100 | 149.63 | 59.19 | 134.74 | 392.64 | 195.77 |
| 9 | *Oe. pratincola* hyb. *immunis* × *Oe. cinerescens* | Susceptible | 100 | 298.65 | 164.41 | 308.56 | 618.02 | 609.91 |
| 13a | *Oe. mississippiensis* × *Oe. cinerescens* | do | 100 | 202.28 | 74.36 | ---------- | 373.59 | 346.69 |
| 14 | *Oe. cinerescens* × *Oe. mississippiensis*, metaclinic hybrid | do | 100 | 234.18 | 80.79 | ---------- | 379.65 | 395.47 |
| 24 | *Oe. pratincola* mut. *nitidissima* | Resistant | 100 | 71.35 | 25.46 | ---------- | 277.37 | 95.25 |
| 25 | do | Susceptible | 100 | 82.33 | 36.49 | ---------- | 308.54 | 137.18 |

TABLE 33.—*Constitution of reciprocal hybrids and mutations of Oenothera, expressed as ratios, sugar being taken as unity.*

| Key No. | Name of strain. | Characteristic of strain. | Total sugar by acid hydrolysis. | Pentosan. | Total nitrogen. | Tannin. | Water soluble acid. | Total ash. |
|---|---|---|---|---|---|---|---|---|
| 1a | Resistant *Oe. pratincola* (a segregate from species hybrid) | Resistant | 100 | 78.65 | 26.83 | 104.45 | 261.12 | 93.67 |
| 2a | Mildewed *Oe. pratincola* (a segregate from species hybrid) | Susceptible | 100 | 134.94 | 69.69 | 66.99 | 398.65 | 253.67 |
| 3a | *Oe. pratincola* hyb. *immunis* | Resistant | 100 | 155.24 | 55.25 | 193.85 | 460.97 | 189.50 |
| 4a | Mildewed *Oe. pratincola* (a segregate from species hybrid) | Susceptible | 100 | 341.00 | 178.34 | 250.23 | 1,010.12 | 611.97 |
| 8 | *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis* | Resistant | 100 | 111.05 | 43.93 | 74.21 | 291.39 | 145.29 |
| 9 | *Oe. pratincola* hyb. *immunis* × *Oe. cinerescens* | Susceptible | 100 | 96.79 | 53.28 | 32.41 | 200.28 | 197.66 |

In *Oenothera* it is well known that whole groups of morphological characters cohere strongly in inheritance. The explanation of the phenomenon is still a matter for investigation. Geo. H. Shull(44) thinks that he has reached a basis for an explanation in his hypothesis that the factorial differences between the various *Oenothera* strains in the congeries of elementary species and hybrids grouped about *Oe. biennis, Oe. pratincola, Oe. lamarckiana,* etc., are located in a single chromosome. Therefore, all factorial complexes are hereditary as a whole, except as crossing-over may occur. A more conservative hypothesis is that of Bartlett and of Cobb and Bartlett,(5, 10) according to whom the peculiarities of inheritance in *Oenothera* need not necessarily be viewed as dependent upon all the mutable factors being located in a single chromosome. On the contrary, several chromosomes may conceivably form a coherent group in the reduction division apart from a residue of other freely segregating chromosomes which are probably the bearers of typically Mendelian characteristics. The hypothesis thus briefly outlined is the one (called the $\alpha$ and $\beta$ hypothesis) which has served for the formulation of the genetical data concerning the strains dealt with in this paper. It has been brought forward subsequently as something new by Lotsy,(29) who calls it the "Kernchimaera" hypothesis.

Without this slight digression the reader might misunderstand my conception of resistance to be that it is due to a complex of factors rather than a single factor. It is consistent with all that is known of the genetics of *Oenothera* for a factor complex to be inherited as a whole. Consequently, the breeding data are sharp and clear in a way that we seldom expect to find in other groups where the factors, which together contribute to immunity, are located in different chromosomes and, therefore, segregate freely from one another when hybridization takes place. In *Oenothera,* when a disease-resistant factor complex has once come about, the peculiarities of inheritance are such that it is not disintegrated even by hybridization; hence, the unusually sharp and simple relationships that have come to light with regard to inheritance. In plants with simpler, freely segregating Mendelian factors, one would expect very few cases in which clear-cut differences with regard to infection would prove to be due to a single Mendelian factor. The results with *Oenothera,* however, lead to the supposition that, if immunity is generally due to contributory and interacting factors, there is every reason why the plant breeder should work according to a consistent program

for the accumulation in a single strain of all factors contributing to disease resistance. In such work the chemical composition of the plant breeder's basic materials might afford him a criterion for guidance during the process of building up a resistant factor complex. The biochemical study of geneticists' materials certainly cannot fail eventually to find important applications.

It is instructive to compare the analytical data for reciprocal hybrids with those for mutations that differ visibly from each other only in resistance. The two members of the several pairs of reciprocal hybrids were usually quite distinct from each other morphologically as well as in resistance. They were selected as material for this study because they show great contrast in immunity, and the analytical data show correspondingly strong contrasts. In the case of the mutations, two types were chosen that were morphologically alike and differed only in resistance. Although called *Oe. pratincola* mut. *nitidissima,* neither was of direct descent from the original mutation, but they were the *nitidissima* types splitting out of crosses with the parent species, *Oe. pratincola.* Factorial recombination brought about forms differing in resistance. Comparison of the analyses shows variations in chemical composition quite parallel with those differentiating the more diverse reciprocal hybrids, but of smaller magnitude.

Another very interesting fact is brought out in the comparison of one member of a pair of reciprocal hybrids with a morphologically identical metaclinic hybrid in the reciprocal cross. To illustrate how these metaclinic plants arise, let us take a hypothetical case of a cross between species A and B, of which the matroclinic, true-breeding first generation hybrids are A′ and B′. Then

$$A \times B \rightarrow A'$$
$$B \times A \rightarrow B'$$

In rare instances, however, the progeny consisting of hybrid A′ may contain individuals of hybrid B′ and, conversely, the progeny consisting of hybrid B′ may contain a few individuals of A′. These plants which appear to be out of place are called metaclinic hybrids by De Vries.(17) In some instances, notwithstanding morphological identity with the majority of plants in the reciprocal cross, they will be different as to immunity. In such cases there are likewise chemical differences, of the same direction for the same constituents, as in the resistant and non-resistant types of *Oe. pratincola* mut. *nitidissima.*

In connection with the relative differences found in chemical composition between susceptible and resistant strains of *Oenothera,* it is of interest to give a brief account of the modes of entrance of the hyphae and haustoria of the Erysipheae into the cells of the host. Salmon(41) states that—

> the ordinary vegetative mycelium consists of very numerous, delicate, white or colorless septate hyphæ, frequently branched and more or less densely interwoven. In all genera, except Phyllactinia, the hyphæ of the vegetative mycelium produce haustoria at intervals which pierce the cuticle and swell out into a bladder-like form in the epidermal cells. These haustoria serve both to attach the fungus to its host and draw nourishment from it.

Smith(45) describes the haustorium of the Erysiphae, in the specific case of *Erysiphe communis* on *Geranium maculatum,* as

> a slender, proximal portion, the neck, penetrating the epidermal wall of the cell, within which it enlarges into a vesicular, distal portion with a thin wall. On the interior, the vesicle is filled with a delicate, spongy protoplasm differing in no visible particulars from the protoplasm in the mycelium.

In *Oenothera,* according to Klaphaak and Bartlett,(27) the mildew *Erysiphe polygoni* DC. grows very superficially and the haustoria of the fungus extend into the epidermal cells. It is, therefore, the epidermal tissue that should be examined if the most nearly perfect correlation of chemical composition with resistance is to be discovered. The composition of the whole leaf can be of significance only if there is a correlation between the composition of the epidermis and the underlying tissues. Such a correlation I have assumed, and the results are so consistent throughout that there is no reason to question the validity of the assumption. If we visualize the epidermal cells as comparatively rich in tannin, sugar, and water-soluble acids, we must bear in mind that either the actual synthesis of these substances occurs in the green cells or else the substances from which they are synthesized come from the green cells. The equilibrium between the chemical content of the epidermal cells and that of the underlying tissues is of such a nature that we must suppose transposition of material to take place whenever the concentration of water-soluble substances changes. The epidermis, unable to carry on fundamental chemical synthesis, must be in the most intimate equilibrium with other tissues, and we should expect differences in epidermal content to be correlated with differences in the leaf as a whole.

The rôle of tannin has been studied extensively, both from the point of view of the metabolism of the host and from that of its influence on parasites. Here we are not concerned with the question whether tannin is regarded as a by-product of metabolism or as a reserve material. The point of importance is whether or not it prevents infection by mildew. The results of the earlier investigations in this regard are conflicting. Cook and Taubenhaus(13) were able to demonstrate that tannin is toxic to the growth of certain fungi, the parasitic forms being more sensitive than the saprophytic. Wehmer(54) found that the resistence of oak wood to dry rot is correlated with the amount of tannin in the wood. On the other hand, Valleau(55) obtained negative results in his attempt to relate tannin to the differences in resistance of varieties of plums to brown rot. Moreover, Willaman and Sandstrom(51) did not find significant differences in the tannin concentration of normal plums and the ones rotted by *Sclerotinia cinerea*. The conflicting results may be due to differences in the localization of tannin in different parts of a plant or plant organ. No fact is more familiar to the geneticist than the existence of factors which determine the distribution in certain cells of pigment, tannin, enzymes, etc. Such factors give rise to the color patterns of flowers and animals. In *Oenothera*, the results show no evidence of disturbance due to such factors, but plums might present entirely different conditions. Tannin localized elsewhere than in the epidermis would have no influence on infection by the fungus which grows superficially in plant tissue. Analysis of a whole fruit might show a high tannin content but, nevertheless, the fruit might have no resistance. When the difference of the tannin content between resistant and susceptible plants fails to correlate with resistance, it may be that the tannin is differently located. The action of tannin as a protective agent is summarized by Thatcher(47) in the following words:

> Either the tannin actually serves as an antiseptic to prevent the growth of the parasitic fungus within the tissues of the host plant, or it assists in the development of a corky layer which "walls-off" the infected area and so prevents further spread of the disease through the tissue.

The fact that tannin coagulates albumin and other proteins, suggests that it may be a protoplasmic poison. When the fungi are grown in synthetic media containing varying amounts of tannin, the harmful effect of the tannin is manifested in the majority of cases. However, if tannin is injurious to fungus protoplasm, why is it not also harmful to the protoplasm of the

host plant? It is very likely that the protective effect of tannin in preventing the growth of parasitic fungi is conditioned by the other substances present in the cell sap. Thus De Dominicis (15) found that the coagulation of egg albumen by tannin can be prevented by the addition of acetic acid or tartaric acid, and that these two acids restore to its original condition an albumin which has been coagulated by tannin. Possibly in the tissues of a plant there are other substances besides the common acids which may serve to protect the protoplasm from the tannin of the same species. It is quite possible, however, that the foreign protoplasm of a parasitic fungus would be adversely affected by the same tannin. In this connection it is interesting to observe that an *Oenothera* that is high in tannin is likewise high in acid. Thus in the resistant strains we have a high tannin content, for which there is evidence of an immunizing function, coupled with a high acidity which is not inconceivably protective to the protoplasm. Thus an entire mechanism for resistance is present. Susceptible strains have a lesser development of the factorial complex for immunity, coupled with high water-soluble nitrogen which probably favors the nutrition of the mildew. One can hardly avoid the conclusion that striking differences in resistance are due to the interaction of various factors.

Most fungi grow best in slightly acid media but, when the concentration of acid is rather high, it inhibits growth. The cell-sap of the leaves of the resistant *Oenothera* is very high in acid which may influence the fungus adversely, quite aside from its possible protective influence in preventing coagulation of the proteins of the host protoplasm. The relation of acid content of the plant to disease resistance has been pointed out by many investigators. Comes(11) found that Rietti wheat, a very resistant variety, has a higher percentage of acid than other, susceptible varieties. Moreover, he also observed that, as the rust resistance of wheats increases with the altitude at which they are grown, their acid content is also correspondingly increased. Averna-Sacca(3) found that American grape and other plants which are highly resistant to the attacks of *Oidium peronospora* and gall mites are characterized by having a very high acid content. Brown(6) reported that the enzyme activity of an extract from *Botrytis cinerea* is inhibited by the addition of a fair amount of acid. The high concentration of acid in the leaves of the resistant *Oenothera* may be correlated with its resistance.

Turning now to the susceptible plants, we find that their nitrogen and ash content is high. Analyses of the constituents

of total nitrogen and ash reveals further that, in the case of nitrogen, the form that predominates is the water-soluble nitrogen which, in turn, has a high concentration of amino acids and of nitrogenous compounds of nonbasic character. The ash contains a great deal of calcium and sulphur.

Zellner(57) found that Ascomycetes contain much protein but that the amount of tannin, if ever present, is extremely low. According to Pfeffer,(38) most fungi that are able to develop, in media containing nitrogen in the form of ammonium nitrate, grow better when supplied with peptone, amides, and other nitrogenous organic compounds. That the mildew on *Oenothera* probably derives its supply of nitrogen mainly from the water-soluble nitrogenous compounds in the leaves is sustained by the following experimental observation. When susceptible and resistant plants are examined at the period of their highest stage of growth, during which time the mildew on their leaves is also very active, the difference of nitrogen content between resistant and susceptible strains lies in the water-soluble nitrogen. In the same plants, examined at the time when they approach senility, the relative difference in nitrogen still persists but the consistent difference is found in the protein nitrogen. There are two possible explanations to account for this. One is that, during infection, the mildew is continually using some of the simple nitrogenous compounds, perhaps some of the amino acids, leaving other amino acids in excess, which are not immediately utilized for the synthesis of proteins. The equilibrium in the host may be looked upon as disturbed by the withdrawal of protein-forming substances in proportions different from those in which they are utilized in protein synthesis. In the senescent leaves, on the contrary, the mildew is no longer growing and the normal metabolism of the plants is not disturbed. The other explanation is that the formation of simple nitrogenous compounds takes place so rapidly during the height of the growth period that protein synthesis lags somewhat behind. In other words, material for protein synthesis is produced much faster than it can be utilized. The second explanation seems to be the more plausible one. Certainly the high nitrogen content of the susceptible plants would seem to have something to do with their infection by the mildew. Otherwise, they would not be so much more susceptible at one time than at another.

Calcium, to which the high ash content of susceptible *Oenothera* is largely due, is present in the living plant in the form of raphides of calcium oxalate. Its presence may indirectly benefit

the mildew by neutralizing some of the acids in the cell sap. However, complicated questions of plant physiology are here concerned, and the suggestion would hardly be made if it were not for the definite correlation of high ash content with low acidity. Bartlett informs me that the most heavily mildewed *Oenothera* plants he has ever seen were on shingle of a river bank at the base of limestone cliffs. The fact that the total ash is considerably higher in the susceptible plants than in the resistant plants indicates that possibly the rate of absorption of the inorganic elements differs considerably. Since the inorganic salts absorbed from the soil together with the carbon dioxide taken in from the atmosphere constitute the raw materials for the synthesis of food in the plant, it seems very suggestive that the causes for the differences in the amount of elaborated constituents can be traced back to differences in salt absorption.

A chemical basis for true immunity does not necessarily imply the presence of some toxic substances in the resistant plants and the complete absence of such substances in the susceptible plants; conversely, it does not imply the presence of some chemotactic substance in the susceptible plants that does not exist in the resistant ones. Differences of the concentration of these substances in the sap, as well as their localization in the tissues of the plant, are two of the main factors to be considered. A resistant plant and a susceptible one may both contain the same toxic substances or chemotactic substances; but, if the amount of such substances present in one or the other is small, or if they are differently located, then they may exercise no harmful or beneficial influence on the fungus. The effect of the concentration of the substances in the cell sap is well shown in the experimental results obtained with the susceptible and the resistant strains of *Oenothera,* while the effect due to difference of location of the substances in the tissues of the plant is purely an expression of opinion arising from the fact that not all other investigators have been able to establish definite correlations between composition (especially with regard to tannin) and resistance to infection.

## SUMMARY AND CONCLUSIONS

1. Chemical analyses of several resistant and susceptible hybrids and elementary species of *Oenothera* were made in order to discover whether or not there were definite correlations between chemical composition and reaction to mildew. The chemical analyses were also extended to other plants which were

found to have strains resistant and susceptible to other forms of powdery mildew.

2. The resistant strains are characterized by being higher in tannin and water-soluble acid. In the majority of cases, they are also higher in crude fiber than the susceptible ones.

3. The susceptible strains are comparatively high in total nitrogen and total ash.

4. The forms of nitrogen that are high in the susceptible strains during infection are the amino acids and nitrogenous compounds of nonbasic character. When the plants are approaching senility, the leaves of the susceptible plants are extremely rich in protein nitrogen.

5. The ash of the susceptible plants is exceedingly high in calcium and sulphur, as compared with that of the resistant plants.

6. All the strains examined, comprising wild elementary species, reciprocal hybrids, and mutations, show the same correlation of chemical composition with disease resistance.

7. The differences in chemical composition are found in both resistant and susceptible strains, before infection and after, and during the period when the leaves of the plants show chlorophyll degradation, indicating that the characteristic features of the resistant and the susceptible strains are constitutional.

8. The chemical analyses made of the susceptible and the resistant plants of *Syringa vulgaris, Desmodium canadensis, Helianthus giganteus,* and *Solidago canadensis* confirm the findings in regard to *Oenothera.* An exception was found in the total ash and water-soluble acid contents of *Helianthus giganteus,* but the plants were wild and may not have been genetically as close as appearance indicated.

9. Resistance in plants is doubtless due to a complex of interacting factors. Factors that increase tannin and acid and that decrease water-soluble nitrogenous compounds tend to build up immunity.

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# APPENDIX

## RECORD OF THE PARENTAGE OF ALL STRAINS OF OENOTHERA USED IN THE EXPERIMENTS

[C always indicates the type strain of *Oenothera pratincola* Bartlett known as "Lexington C." Similarly D indicates *Oe. numismatica* Bartlett, known as "Lexington D." See Bartlett (4) 1915.]

0847[1] = (C–52–6–25–1–41 *typica* × (C–22–15 × C–22–10) 1–25–34 *simulans*) 7–19 × (CD–9–49–6 *typica* × CD–9–9–46 *simulans*) 2–33–1 = resistant *Oe. pratincola* (a segregate from species hybrid).

(= reciprocal of 0822.)

1827 = 0847–4.

2960 = 0847–4–10.

0822 = (CD–9–49–6 *typica* × CD–9–9–46 *simulans*) 2–33–1 × (C–52–6–25–1–41 *typica* × (C–22–15 × C–22–10) 1–25–34 *simulans*) 7–19 = mildewed *Oe. pratincola* (a segregate from species hybrid).

(= reciprocal of 0847.)

1824 = 0822–26.

2959 = 0822–26–34.

0846 = (C–52–6–25–1–41 *typica* × (C–22–15 × C–22–10) 1–25–34 *simulans*) 7–21 × (CD–9–49–6 *typica* × CD–9–9–46 *simulans*) 2–33–22 = *Oe. pratincola* hyb. *immunis*.

(= reciprocal of 0823.)

[1] These numbers represent "culture number" of the strain; that is, the number under which a progeny was grown in the garden.

1826 = 0846–2.
2956 = 0846–2–31.
0823 = CD–9–49–6 *typica* × CD–9–9–46 *simulans* } 2–33–22 × C–52–6–25–1–41 *typica* × C–22–15 × C–22–10 } 1–25–34 *simulans* } 7–21 = mildewed *Oe. pratincola* (a segregate from species hybrid).
(= reciprocal of 0846.)
1825 = 0823–5.
2954 = 0823–5–61.
0830 = Cartersville 13d–1–22–24–5–6 × CD–9–49–6 *pratincola typica* × CD–9–9–46 *simulans* } 2–33–23 = *Oe. mississippiensis* × *Oe. pratincola* hyb. *immunis*.
(= reciprocal of 0830.)
1804 = 0830–7.
0820 = CD–9–49–6 *pratincola typica* × CD–9–9–46 *simulans* } 2–33–23 × Cartersville 13d–1–22–24–5–6 = *Oe. pratincola* hyb. *immunis* × *Oe. mississippiensis*.
(= reciprocal of 0830.)
1805 = 0820–36.
0829 = Cartersville 13d–1–22–24–5–7 × CD–9–49–6 *pratincola typica* × CD–9–9–46 *simulans* } 2–33–23 = *Oe. mississippiensis* × *Oe. pratincola* hyb. *immunis*.
(Similar to 0830.)
1818 = *cinerescens* (2) 9–$3_{16}$–5–20–1 × CD–9–49–6 *pratincola typica* × CD–9–9–46 *simulans* } 2–33–17 } 60 = *Oe. cinerescens* × *Oe. pratincola* hyb. *immunis*. (Matroclinic in morphology.)
(= $F_1$ of reciprocal cross of parents of 1819.)
2966 = 1818–75.
1819 = CD–9–49–6 *pratincola typica* × CD–9–9–46 *simulans* } 2–33–17 × *cinerescens* (2) 9–$3_{16}$–5–20–1 } 4 = *Oe. pratincola* mut. *immunis* × *Oe. cinerescens*. (Matroclinic in morphology.)
(= $F_1$ of reciprocal cross of parents of 1818.)

2963 = 1819–18.

0838 = *"biennis Chicago"* 2–20–3–1 × CD–9–49–6 *pratincola typica* × CD–9–9–46 *simulans* (2–33–19) = *Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *immunis.*

(= reciprocal of 0821.)

0821 = CD–9–49–6 *pratincola typica* × CD–9–9–46 *simulans* (2–33–19) × *"biennis Chicago"* 2–20–3–1 = *Oe. pratincola* hyb. *immunis* × *Oe. "biennis Chicago."*

(= reciprocal of 0838.)

0814 = *cinerescens* (2)–9–$3_{16}$–5–20–3 × Cartersville 13d–1–22–24–5–5 = *Oe. cinerescens* × *Oe. mississippiensis.*

(= reciprocal of 0827.)

1802 = 0814–64 = *Oe. cinerescens* × *Oe. mississippiensis* (metaclinic).

2958 = 0814–64–27.

0827 = Cartersville 13d–1–22–24–5–5 × *cinerescens* (2)–9–$3_{16}$–5–20–3 = *Oe. mississippiensis* × *Oe. cinerescens.*

(= reciprocal of 0814.)

1800 = 0827–12.

2957 = 0827–12–12.

0299 = C–52–6–31–73–19–6 *typica* × (C–52–6–31–72–3 *typica* × *reynoldsii typica debilis* 56–21) (9) (2) = *Oe. pratincola* × *Oe. reynoldsii* (*Oe. pratincola* hyb. *amycosa.*)

(Similar in genetic constitution to reciprocal of 0177.)

0177 = *reynoldsii typica debilis* 56–21 × C–52–6–31–73–3 (6–4) = *Oe. reynoldsii* × *Oe. pratincola.*

0842 = *"biennis Chicago"* 2–20–3–2 × C–52–6–25–1–41 *typica* × (C–22–50 × C–22–10) 1–25–34 *simulans* (7–22) = *Oe. "biennis Chicago"* × *Oe. pratincola* hyb. *rubricalyx.*

(= reciprocal of 0849.)

1816 = 0842–4.

2962 = 0842–4–10.

0849 = C–52–6–25–1–41 *typica* × (C–22–15 × C–22–10) 1–25–34 *simulans* } 7–22 × "*biennis Chicago*" 2–2–3–2 = *Oe. pratincola* hyb. *rubricalyx* × *Oe.* "*biennis Chicago.*"
(= reciprocal of 0842.)

1817 = 0849–8.

2961 = 0849–8–12.

0831 = Cartersville 13[d]–1–22–24–5–3 × "*biennis Chicago*" 2–20–3–1 = *Oe. mississippiensis* × *Oe.* "*biennis Chicago.*"
(= reciprocal of 0839.)

1806 = 0831–10.

0841 = "*biennis Chicago*" 2–20–3–1 × Cartersville 13[d]–1–22–24–5–3 = *Oe.* "*biennis Chicago*" × *Oe. mississippiensis.*
(= reciprocal of 0831.)

0839 = "*biennis Chicago*" 2–2–3–2 × Cartersville 13[d]–1–22–24–5–8 = *Oe.* "*biennis Chicago*" × *Oe. mississippiensis.*
(= similar to 0841.)

1807 = 0839–2.

0833 = Cartersville 13[d] 1–22–24–5–6 × CD–52–6–25–1–41 *typica* × (C–22–15 × C–22–10) 1–25–34 *simulans* } 7–22 = *Oe. mississippiensis* × *Oe. pratincola* hyb. *rubricalyx.*
(= reciprocal of 0848.)

1810 = 0833–6.

0848 = C–52–6–25–1–41 *typica* × (C–22–15 × C–22–10) 1–25–32 *simulans* } 7–22 × Cartersville 13[d]–1–22–24–5–6 = *Oe. pratincola* hyb. *rubricalyx* × *Oe. mississippiensis.*
(= reciprocal of 0833.)

1811 = 0848–60.

1346 = E–5–208–1–182 *nitidissima* × E–43–74–21 *typica* } 7–1–10 = *Oe. pratincola* mut. *nitidissima.* (Resistant.)

1348 = same as 1346.

1347 = E–5–208–1–182 *nitidissima* × E–43–74–21 *typica* } 7–1–11 = *Oe. pratincola* mut. *nitidissima.* (Mildewed.)

1349 = same as 1347.

199 = D–1–11–24–28–45–34–9–25 ..... = *Oe. numismatica.*

1191 = CD–9–9–42–31–572–1–11 ..... = *Oe. pratincola* mut. *simulans.*

1192 = (C–22–15 × C–22–10) 1–24–2–29–9–4 ..... = *Oe. pratincola* mut. *simulans rubricalyx.*

1829 = (CD–9–49–6 *typica* ..... × CD–9–9–46 *simulans* .....) 2–33–1–8 .... = *Oe. pratincola* hyb. *immunis.*

2953 = 1829–36.

1231 = (*reynoldsii typica debilis* 56–21 × C–52–6–31–73–3 *typica* .....) 6–7–11 ..... = *Oe. reynoldsii* × *Oe. pratincola.*

167 = C–52–6–25–1–43–2–22–4 *typica* ..... = *Oe. pratincola* (susceptible.)

1828 = Cartersville 13[d]–1–22–24–5–9–7 ..... = *Oe. mississippiensis.*

1195 = (C–52–6–25–1–41 *typica* ..... × (C–22–15 × C–22–10) 1–25–34 *simulans rubricalyx* .....) 1–7–3 = *Oe. pratincola* hyb. *rubricalyx.*

1234 = 89–85–40–46–44–22–11 *typica reynoldsii* ..... = *Oe. reynoldsii.*

2950 = "*biennis Chicago*" 2–20–3–1–1–2 ..... = *Oe.* "*biennis Chicago.*"

2952 = (C–52–6–25–1–4 *typica* ..... × (C–22–15 × C–22–10) 1–25–34 *simulans* ..) 7–19–21–27 = *Oe. pratincola* hyb. *rubricalyx.*

# CHEMOTHERAPEUTIC EXPERIMENTS WITH CHAULMOOGRA AND ALLIED PREPARATIONS

## III. THE DISINFECTING POWER OF THE VAPORS OF VEGETABLE OILS TOWARD ACID-FAST BACTERIA

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The results of previous experiments demonstrated the growth-inhibiting effect upon acid-fast bacteria of certain vegetable oils. It seemed to be of interest to arrange experiments so as to ascertain to what extent, if at all, these oils (produced, as they are, by plants in nature and some of them widely used in every-day life) contribute to the natural disinfection of objects exposed to the atmosphere, which is filled with their volatile constituents or secondary products.

Only those oils are included, therefore, in these tests which showed inhibitory activity on previous occasions. Since in these experiments direct contact was prevented between the oils tested and the inoculated bacteria, only those constituents and secondary products came into action which evaporate in sufficient amount at incubation temperature (37° C.); that is to say, gases evolved and constituents of low boiling point.

A great many of the oils previously tested had a considerable inhibitory effect upon *Bacillus tuberculosis* when brought into direct contact with the freshly inoculated culture. It was expected that, in some cases at least, it might be possible to reach a decision as to whether in a case of a given oil the inhibition of growth is due to the direct action of the stable constituents or to the volatile constituents and secondary products given off during the process of drying.

The following technic was employed in these experiments: Glycerine-meat-infusion-agar slants were planted with a well-growing strain of *B. tuberculosis*, human type, a small loopful

[1] Member, Philippine Leprosy Research Board.

of growth being transferred and distributed well over the surface of the slant. About 0.1 cubic centimeter of the substance to be tested was placed on the inner end of the cotton plug. The cotton plug was immediately replaced in the culture tube and sealed with paraffine. From the time the wetted cotton plug was replaced the culture tubes were handled and incubated in an inverted position (plug down). Readings were made two weeks and, again, four weeks after incubation. For comparison freshly inoculated cultures of *Staphylococcus, Vibrio choleræ, Bacillus dysenteriæ,* and *B. coli* were similarly treated.

TABLE 1.

| Name of oil. | *Bacillus tuberculosis.* | *Staphylococcus.* | *Vibrio choleræ.* | *Bacillus dysenteriæ.* | *Bacillus coli.* |
|---|---|---|---|---|---|
| Bergamot | =<br>= | + | +<br>Inh | + | + |
| *Caryophyllum* | =<br>= | + | + | + | + |
| Cashew | +<br>+ | + | + | + | + |
| Cedar | +Inh<br>+Inh | + | + | + | + |
| Ceara rubber | +<br>+ | + | + | + | + |
| Cinnamon | =<br>= | +<br>Inh | +<br>Inh | +<br>Inh | +<br>Inh |
| *Citrus microcarpus* | =<br>= | + | — | + | + |
| Coconut | +<br>+ | + | + | + | + |
| Copaiba | +<br>+ | + | + | + | + |
| *Dacrydium* | =<br>= | + | — | + | + |
| Eucalyptol | =<br>= | + | — | + | + |
| Palomaria | +Inh<br>+Inh | + | + | + | + |
| *Pinus sylvestris* | =<br>= | — | + | + | + |
| *Pittosporum* | =<br>= | — | + | + | + |
| Turpentine | =<br>= | +<br>Inh | +<br>Inh | + | + |
| Vetiver | +Inh<br>+Inh | + | + | + | + |
| Chaulmoogra (Japan) | +<br>+ | + | + | + | + |
| *Hydnocarpus alcalæ* | +<br>+ | + | + | + | + |
| *Hydnocarpus subfalcata* | +<br>+ | + | + | + | + |
| *Hydnocarpus venenata* | +<br>+Inh | + | + | + | + |
| *Hydnocarpus wightiana* | +<br>+ | + | + | + | + |
| *Gynocardia odorata* | +<br>+ | + | + | + | + |

The following abbreviations are used in Table 1:

$+$ = Growth same as untreated control.

$\overset{+}{+}$ = Growth same as untreated control in two and four weeks.

$\overset{-}{-}$ = No growth in two and four weeks.

$-$ = No growth in forty-eight hours.

$+$ Inh = Scanty growth.

$\overset{+\ \text{Inh}}{+\ \text{Inh}}$ = Growth in two and four weeks scantier than controls.

This experiment produced evidence that vapors of oils containing acids of the chaulmoogric series show no disinfecting effect whatsoever. On the other hand, a great many essential oils, such as bergamot, *Caryophyllum,* cinnamon, *Citrus microcarpus, Dacrydium,* eucalyptol, *Pinus sylvestris, Pittosporum,* and turpentine evolved a sufficient amount of volatile and active constituents to inhibit completely the growth on agar of one loopful of viable tubercle bacilli in the proportion of 0.1 cubic centimeter to 15 cubic centimeters of closed air space at 37° C.

No evidence was adduced in these experiments to show that fixed vegetable oils evolve at the low temperature of 37° C. a sufficient amount of secondary volatile products to cause inhibition of growth of acid-fast bacteria in vitro.

## CONCLUSIONS

The vapors of chaulmoogra and *Hydnocarpus* oils show no disinfecting effect upon acid-fast bacteria.

The volatile constituents of certain essential oils show high disinfecting activity with a strong indication of selectivity toward acid-fast bacteria.

# MONILIA PSILOSIS ASHFORD IN SEVERE ANÆMIA ASSOCIATED WITH THE SPRUE SYNDROME

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ONE PLATE

The persevering work of Ashford(1–8) in Porto Rico during the past few years, establishing "sprue" on a sound laboratory basis as an infectious disease due to the causative microörganism *Monilia psilosis*, has not yet been fully accepted. The condition has been so long a bone of contention, particularly among clinical investigators, that it is not surprising perhaps that this should be so. This unsettled state of affairs is, therefore, my justification for publishing these notes on the occurrence of a *Monilia*, which fulfills the cultural and serological requirements and presents the morphological characteristics of Ashford's *Monilia*, in certain fatal cases of anæmia associated more or less definitely with the syndrome recognized clinically as sprue.

My attention was first called by Major J. Earle Ash, of the Sternberg General Hospital staff (United States Army hospital), in Manila, to a small group of cases presenting an anæmia of an extraordinary degree, resembling pernicious anæmia in its severity and the rapidity of its course, but showing very few of the morphological changes of the blood commonly found in that condition (Plate 1, fig. 1).

The red cell count in the typical advanced case is under one million, and the leucocyte count is correspondingly diminished to about three thousand or less per cubic millimeter. The differential count shows a marked relative lymphocytosis, often as high as 85 per cent, while the polymorphonuclear neutrophiles are proportionately decreased. A few atypical large mononuclear cells are occasionally seen and, rarely, a basophilic or eosinophilic polymorphonuclear can be found. The platelets are absolutely decreased to from 100,000 to 150,000, as a general rule. On careful examination of the red cells, with the usual

triacid strains (Wright's, Jenner's, Giemsa's) it is noted that, while there is a slight tendency to microcytosis, there is seldom an appreciable poikilocytosis. Furthermore, the cells, while tremendously diminished in actual numbers, when examined under the microscope appear essentially normal. The hæmoglobin percentage is, of course, low, usually from 30 to 40, thus giving a high color index.

Another noticeable feature of the blood film is the absence of any evidence of marrow activity; there are usually no nucleated red cells, or at the most a very occasional one; there is no stippling or polychromatophilia; and the reticulated red cell count ordinarily is within or under normal limits. The fragility of the red cells as tested in varying dilutions of hypotonic salt solution shows a very slight increase in resistance, moving never more than two tubes. In short, the blood findings are those we familiarly associate with the so-called idiopathic (sui generis) aplastic anæmia, the literature of which I collected in 1918.(17)

Another striking feature of the disease is that, at least in the group of cases seen by Ash, it always occurs in Europeans or Americans who have lived in the Tropics a number of years. and that it is always associated with the syndrome commonly recognized as sprue; namely, a chronic intestinal disturbance characterized typically by enormous frothy, pale stools, nausea, gaseous eructations, abdominal distress, a sore tongue somewhat like that of pernicious anæmia, and finally emaciation and death.

With this picture in mind it occurred to me that here was an opportunity perhaps to secure evidence concerning the production of this condition. The term "sprue," as commonly used in the Philippine Islands and elsewhere in the Tropics, is unquestionably a much-abused one. Sprue and dengue are household words to every white inhabitant of the Tropics. Dengue is apt to be the casual diagnosis given to most acute infections having a duration of not more than a week or ten days, in which some definite causative agent is not found or recognized. Sprue, similarly, is the name applied to many of those chronic or intermittent intestinal disturbances in which amœbæ or dysentery bacilli cannot be found. The importance, therefore, of being able to establish sprue on the basis of a true infectious disease, capable of recognition by laboratory methods, either cultural or serological, or both, is obvious. Ashford (1–8) has done the pioneer work in that field in Porto

Rico; but, as he himself comments, it should be corroborated in other parts of the world. That his work has not as yet gained general acceptance may be indicated by a recent paper of Bovaird,(10) of New York.

This paper deals with several cases presenting the picture of severe anæmia associated with the chronic intestinal disturbances of sprue. The cases in every way coincide with those seen earlier with Ash before this work was begun, and lead me to believe that they form a perfectly definite clinical entity. All the cases, with a single exception, occurred in Europeans or Americans who had lived in the Tropics several years. The exception was the case of a Filipino, 30 years of age; but, in spite of slight differences in the clinical picture, I feel that it probably belongs in this group.

The incentive to follow up this problem came from the incidental finding of a *Monilia* from the tongue scrapings and stool of one of these severe cases of anæmia. On isolating this organism it was interesting to note that it conformed in morphological and cultural characteristics to *Monilia psilosis* as described by Ashford. This in itself was of great significance as, to my knowledge, this particular species had not been reported from the Philippines previously. Accordingly, as other cases appeared, efforts were made to isolate the same *Monilia*, and thus far they have been strikingly successful. Interestingly enough, in a large series of controls (including Caucasians, Chinese, and Malays), no definitely comparable *Monilia* has been found; and in those cases where some other *Monilia* was present, the serological reaction or cultural characteristics excluded its specific nature.

It has long been conceded, I believe, by most investigators of sprue, that yeasts or fungi of one sort or another are usually found in connection with these cases. The chief discussion has always been whether the yeast was causative or merely coincidental.

The cases on which this work is based are outlined briefly below, only the essential points in the history and laboratory findings being given.

CASE M.S., I

A Spanish woman, aged 52, born in Guam, and who had lived in the Philippines for the past twenty years except for one visit to Spain two years ago, had had intermittent attacks of diarrhœa for about five years. While in Spain her condition

showed some improvement. Shortly after her return to Manila eight months ago, she developed a marked recurrence of her diarrhœal attacks. These increased in frequency and were accompanied by nausea, gaseous eructations, and abdominal distress. During the past three months she grew weak, lost over 30 kilograms in weight, and became very pale. Her tongue was so painful at times that she refused to eat because of the discomfort of mastication and deglutition. She had been treated by several physicians without any marked improvement, and finally consented to enter the hospital only as a last resort. During her stay of about a month her condition grew progressively worse, and in spite of transfusion and very careful treatment she gradually lost strength and died. No autopsy could be obtained.

The laboratory examinations in brief follow:

TABLE 1.—*Blood examination of M.S., I.*

| Date. | Red cells per cubic millimeter. | Leucocytes per cubic millimeter. | Tallquist hæmoglobin. | Differential count. | | | |
|---|---|---|---|---|---|---|---|
| | | | | Polymorphonuclears. | Lymphocytes. | All others. | Nucleated red cells. |
| | | | *Per cent.* | | | | |
| November 15 | 1,264,000 | 3,200 | 40 | 33 | 61 | 6 | 0 |
| November 20 | 1,056,000 | 2,800 | 30 | 26 | 70 | 4 | 0 |
| November 25 | 976,000 | 2,300 | 30 | 24 | 69 | 7 | 2 |
| November 30 | 784,000 | 2,400 | 20 | 16 | 81 | 3 | 0 |
| December 5 | [a] 2,832,000 | 4,900 | 50 | 44 | 47 | 9 | 3 |
| December 10 | 2,112,000 | 3,400 | 50 | 39 | 54 | 7 | 1 |
| December 15 | 1,656,000 | 2,800 | 40 | 38 | 54 | 8 | 1 |

[a] Post transfusion.

*Blood films.*—All smears showed a typical aplastic picture with slight microcytosis, no polychromatophilia, stippling, or reticulation, and very little poikilocytosis. The leucocytes were small, mature in type, with a progressive preponderance of lymphocytes, relatively. Practically no nucleated red cells were seen. The platelets were definitely diminished, ranging from 100,000 to 125,000. The fragility, as tried on two occasions, read from 0.40 to 0.22.

*Cultures.*—Tongue scrapings on repeated examinations yielded a characteristic mycelium which showed good growth on Sabouraud's medium after forty-eight to seventy-two hours and which conformed to the requirements of Ashford's *Monilia psilosis,* producing acid in dextrose, levulose, galactose, and

saccharose, causing no change in litmus milk, or lactose, and having inverted pine-tree appearance in gelatine media. Similarly, cultures from the large, soft, frothy, greenish gray stools gave an identical *Monilia* on several occasions.

*Complement fixation.*—Positive with an aqueous extract of *Monilia psilosis* as antigen.

CASE M.S., II

Mrs. A., an American, has lived constantly in Manila for the past twelve years. She has not felt really well since an attack of amœbic dysentery three years ago. During the past four months she has had "bilious" attacks with nausea, abdominal pain, a good deal of gas, and alternating periods of diarrhœa and constipation. Her mouth and tongue have been painful for the past six weeks. She has completely lost her appetite, is pale in appearance, and has lost some weight.

*Blood examination.*—The blood showed a hæmoglobin of 45 per cent, a red cell count of 2,864,000, and the typical hypoplastic appearance of the bone marrow as evidenced by the stained film. The leucocytes numbered 4,400, with 38 per cent polynuclears, 59 per cent lymphocytes, and 3 per cent eosinophiles. The platelets numbered 160,000. The fragility was normal, 0.42 to 0.28.

*Cultures.*—From both mouth and stool, positive for *Monilia psilosis*. Stools were pale, soft, and somewhat frothy.

*Complement fixation.*—Positive.

Patient returned to the United States following transfusion, and the end result is not known at present writing.

CASE M.S., III

Philippine General Hospital No. 105272, D.M., aged 30, Filipino. The history differs from the others in an insidious onset of four years' duration, the chief symptoms of which were progressive weakness and pallor accompanied by intermittent œdema of the legs, but no loss of weight. His appetite has not been impaired. There has been only a vague history of gastrointestinal disturbance. From the history alone a diagnosis of hookworm disease might be suggested tentatively; but, in view of the laboratory findings (a positive stool for *Monilia*, the positive complement fixation, the absence of parasites or ova in the stools, and the typical blood picture of this group of cases), it seems more probable that it should be classified as an abortive form of the disease. This patient

developed a lobar pneumonia two days after admission to the hospital and died. The autopsy findings are consistent with the diagnosis.

*Blood examination.*—On admission the blood showed a red cell count of only 630,000, a white cell count of 3,200, and a hæmoglobin of approximately 30 per cent. The differential count gave 52 per cent polymorphonuclears, 47 per cent lymphocytes, and 1 per cent eosinophiles. The blood film presented the typical picture of nearly normal appearing red cells, diminished in number, but showing very slight changes in size or shape, no polychromatophilia, stippling, or reticulation, and no normoblasts.

*Cultures.*—Stools were positive for *Monilia psilosis* and *M. albicans.*

*Complement fixation.*—Positive.

CASE M.S., IV

Mrs. F., an American, 45 years of age, who had suffered from "chronic dysentery" for several years, finally sought medical advice for marked asthenia, loss of weight, and general poor health. A blood smear was taken and a red cell count made. The red cells numbered between 800,000 and 900,000. The smear I had an opportunity to examine, through the courtesy of her attending physician. It showed the same lack of anisocytosis and poikilocytosis previously commented on, and gave no evidence of marrow activity, such as stippling, polychromatophilia, reticulation, or the presence of nucleated red cells. The differential count showed a preponderance of lymphocytes, 71 per cent. Cultures or serum were unfortunately not obtained from this case. Transfusion was recommended and refused, and the patient died on her way back to the United States.

CASE M.S., V

Mrs. B., a Spanish-American mestiza, 48 years of age, a resident of Manila for over twenty years, entered the hospital complaining of weakness, loss of weight, chronic diarrhœa, and a sore mouth. Her history dated back about two years, with an acute exacerbation of only about three months' duration. Examination of the blood gave a red cell count of 2,164,000 per cubic millimeter, a white cell count of 3,800 per cubic millimeter, a hæmoglobin of 40 per cent by Tallquist, a differential white count with 32 per cent polymorphonuclear leucocytes, 61 per cent lymphocytes, and 7 per cent miscellaneous leucocytes,

a platelet count of 200,000, a fragility test reading from 0.40 to 0.24 per cent sodium chloride, and a stained film presenting the previously noted peculiarities.

*Cultures.*—From stool, positive for *Monilia psilosis*. Cultures from tongue, also positive.

*Complement fixation.*—Positive.

CASE M.S., VI

Mr. H., an American ex-soldier, 35 years of age, who has lived in the Philippines for eight years, came to the hospital dispensary complaining of weakness and chronic diarrhœa. He has a history of previous amœbic dysentery and malaria, three and two years ago, respectively. A routine examination of his blood and stool revealed a moderate anæmia and the presence of several imperfect fungi, among them *Monilia psilosis*. His red cell count was 2,864,000 per cubic millimeter, his leucocytes numbered 4,900 per cubic millimeter, and his platelets were approximately 300,000 per cubic millimeter. His hæmoglobin was around 55 per cent by Tallquist. The differential count in this case was nearly normal, with 58 per cent polymorphonuclear leucocytes, 38 per cent lymphocytes, and 4 per cent eosinophiles. The blood smear showed the same general absence of bone marrow activity. In other words, the anæmia was more of an aplastic than a secondary type, morphologically. The serological reaction with a *Monilia* antigen was positive, and the organism was isolated from both the mouth and the stools. The case was a relatively mild one, was treated by transfusion, and three months later he appeared much improved, with a red cell count of 4,500,000 and no symptoms.

CASE M.S., VII

Mr. E., an American, 40 years of age, for about two years has complained of vague or mild intermittent gastrointestinal disturbances. Recently he has felt worse, tires easily, has lost interest in his work, is weak, and is losing weight. He had not particularly noticed any pallor, and his tongue and mouth have not been painful.

*Blood examination.*—The blood gave a red cell count of 2,120,000 per cubic millimeter, a white cell count of 5,800 per cubic millimeter, and a hæmoglobin percentage of 40. The platelets were not counted, but from smear preparation appear decreased in numbers. The differential count is: Polymorphonuclear neutrophiles, 51 per cent; lymphocytes, 42 per cent; eosinophiles, 4 per cent; and large mononuclears, 3 per cent.

The stained film shows findings similar to those noted previously; namely, a relatively normal appearance of what red cells are present, a slight tendency toward microcytosis, and absence of polychromatophilia, stippling, and nucleated red cells.

*Cultures.*—Positive from stool for both *Monilia psilosis* and *M. albicans;* from the tongue, *M. albicans* alone was obtained.

*Complement fixation.*—Doubtfully positive.

CASE M.S., VIII

Mr. O., an American, about 45 years of age, who has lived in the provinces of the Philippines for many years, and has suffered from typhoid, dysentery, malaria, dengue, and miscellaneous minor infections. He had felt as well as usual up to about two years ago when he had the typical history of sprue and was given dietetic treatment for about six months and felt much better. Was unable to keep this diet up because of the inaccessibility of the places where his work took him. Noticed increasing discomfort, with frequent attacks of nausea, "indigestion," and diarrhœa, developed sore tongue and throat, lost his appetite, and began to lose weight. He kept at work, however, until he was unable to rise from his bed because of weakness, and was taken to the hospital for treatment. He is a large man but, with the loss of over 30 kilograms, his skin hangs around his arms and legs in great folds. His color is a sallow, ashen gray. He vomits everything, including water, and is being kept alive by rectal feedings and intravenous injections.

*Blood examination.*—The red cell count is 584,000 per cubic millimeter; the white cells number 2,100; the hæmoglobin is inestimable, but by Tallquist about 15 per cent. The differential count shows a polynuclear neutrophilic percentage of only 11, while the lymphocytes show 83; there are 2 per cent eosinophilic leucocytes and 4 per cent large mononuclears. The stained film presents more marked changes than is usual, with perfectly definite although relatively slight variation in the size and morphology of the red cells. Achromia is more prominent likewise, and an occasional polychromatophilic cell is seen. No increase in reticulation or of stippling is noted, and no nucleated red cells are found. The platelets are reduced to under 100,000 per cubic millimeter. The fragility is decreased noticeably, beginning at 0.38 per cent sodium chloride and not being complete until 0.20 per cent.

*Cultures.*—A profuse growth of *Monilia psilosis* was secured from both mouth and stool specimens.

*Complement fixation.*—Positive.

In Table 2 we find a composite picture of the essential laboratory findings of these eight cases comprising the basic material for this paper. In referring to the blood pictures it is well to remember that the normal leucocyte picture among Filipinos is quite different from the one we are accustomed to in the United States. Guerrero and Sevilla(13) have reported that the normal polynuclear neutrophiles amount to 51.6 per cent; the lymphocytes, 34.5 per cent; and the eosinophiles, 11.2 per cent in a differential count. Whether this is true of the Tropics in general and therefore applicable to Europeans or Americans of long residence in the Philippines I am not prepared to state. It is unquestionably true that the high incidence of parasitic infection tends to raise the eosinophilia average. The sum of the reported leucocyte incidence of 51.6 per cent neutrophiles and 11.2 per cent eosinophiles is not very far from the average polynuclear count among Americans. This point I raise merely to exclude any possible criticism in regard to the standards of comparison. In general, it is distinctly my impression that the lymphocyte count averages from 10 to 20 per cent higher among Filipinos than among Americans, at the expense of the polynuclears. In this group of cases we see a distinct tendency toward a relative lymphocytosis, although an actual leucopenia is present. How much this is affected by the climatic or environmental factors, and how much by infection, are questions largely open to argument and personal opinion. Personally, I feel it is principally due to the infection, as I have not noted it generally among other cases.

## CULTURAL STUDIES

The methods employed in obtaining the *Monilia* were essentially those of Ashford, his technic having been followed with only minor modifications.

Scrapings from the tongue mucosa and material from fæcal suspensions were plated on a modified Sabouraud's medium. This consists of a strongly acid (2.5 + per cent), 4 per cent maltose agar to which a small amount of glycerine had been added. The only advantage which the glycerinating of the medium seemed to accomplish was to secure a more rapid growth of the organisms, and thus much time was saved in the prelimi-

TABLE 2.—*Composite table of laboratory examinations.*

| Case. | Red cells per cubic millimeter. | White cells per cubic millimeter. | Platelets per cubic millimeter (in thousands). | Tallquist hæmoglobin. | Differential count. | | | Stained film. | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Polymorphonuclears. | Lymphocytes. | All others. | Anisocytosis. | Poikilocytosis. | Polychromatophilia. |
| | | | | *Per cent.* | | | | | | |
| M.S., I[a] | 784,000 | 2,400 | 100–125 | 20 | 16 | 81 | 3 | +— | 0 | 0 |
| M.S., II | 2,864,000 | 4,400 | 160 | 45 | 38 | 59 | 3 | +— | 0 | 0 |
| M.S., III | 630,000 | 3,200 | ---------- | 30 | 52 | 47 | 1 | + | +— | 0 |
| M.S., IV | 850,000 | ---------- | ---------- | ---------- | 26 | 71 | 3 | +— | 0 | 0 |
| M.S., V[a] | 2,164,000 | 3,800 | 200 | 40 | 32 | 61 | 7 | +— | 0 | 0 |
| M.S., VI | 2,864,000 | 4,900 | 300 | 55 | 58 | 38 | 4 | 0 | 0 | 0 |
| M.S., VII | 2,120,000 | 5,800 | ---------- | 40 | 51 | 42 | 7 | +— | 0 | 0 |
| M.S., VIII[a] | 584,000 | 2,100 | 80–100 | 15 | 11 | 83 | 6 | ---------- | ---------- | ---------- |
| Average | 1,607,500 | 3,800 | ---------- | 35 | 35.5 | 60.3 | 4.2 | ---------- | ---------- | ---------- |

| Case. | Stained film. | | | Fragility hæmolysis. | | Complement fixation with *Monilia* antigen. | Mouth cultures. | | Stool cultures. | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Stippling. | Reticulation. | Nucleated red cells per 100 white blood count. | Begins. | Ends. | | *Monilia.* | Other yeasts, etc. | *Monilia.* | Other yeasts, etc. |
| M.S., I[a] | 0 | OK | 0–1 | 0.40 | 0.20 | ++ | + | + | + | + |
| M.S., II | 0 | OK | 0 | 0.42 | 0.28 | ++ | + | + | + | + |
| M.S., III | 0 | OK | 1 | (b) | (b) | (c) | (c) | (c) | (c) | (c) |
| M.S., IV | 0 | OK | 0 | (c) | (c) | (c) | (c) | (c) | (c) | (c) |
| M.S., V[a] | 0 | OK | 0 | 0.40 | 0.24 | ++ | + | + | + | + |
| M.S., VI | 0 | OK | 0 | 0.44 | 0.28 | + | + | — | + | — |
| M.S., VII | 0 | OK | 1 | (c) | (c) | +— | — | + | + | + |
| M.S., VIII[a] | 0 | OK | 0 | 0.38 | 0.20 | + | + | + | + | + |
| Average | ---------- | ---------- | P. 51.6 | ---------- | ---------- | ---------- | ---------- | ---------- | ---------- | ---------- |

[a] The breeding and clotting time in three cases showed no change. [b] Unsatisfactory. [c] Not examined.

nary isolation. All suspicious cultures were fished after twenty-four hours and planted in duplicate on Sabouraud's and the glycerinated Sabouraud's; the former to serve as the permanent culture, the latter to be used for transplants. After another twenty-four to forty-eight hours the organism was planted (*a*) in the various sugar fermentation media, (*b*) in litmus milk, and (*c*) in stab culture on Hiss' semisolid medium, a very satisfactory substitute for pure gelatine in the Tropics. Absolute identity of the *Monilia* species was checked by the appearance of massive cultures on Sabouraud's medium after two to three months, as the early cultures are not always readily differentiated even by their cultural reactions in milk and carbohydrates (Plate 1, fig. 2).

I present a table showing the reactions with twenty of the familiar laboratory sugars. As no additional information was obtained by the use of any but a few simple sugars, the complete examination was given up after the first three cases had been tested.

TABLE 3.—*Carbohydrate reactions with Monilia psilosis.*

| Sugar used. | 1 | 2 | 3 |
|---|---|---|---|
| Adonite | — | — | — |
| Arabinose | — | — | — |
| Dextrose | +++ | +++ | +++ |
| Dulcit | — | — | — |
| Galactose | ++ | + | + |
| Inosit | — | — | — |
| Inulin | — | — | — |
| Lactose | — | — | — |
| Levulose | +++ | ++ | ++ |
| Mannit | — | — | — |
| Mannose | + | ?+ | ?+ |
| Maltose | +++ | +++ | +++ |
| Melozitose | — | — | — |
| Quercit | — | — | — |
| Raffinose | — | — | — |
| Rhamnose | — | — | — |
| Saccharose | ++ | ++ | + |
| Sorbit | — | — | — |
| Trehalose | — | — | — |
| Xylose | +— | — | +— |

In routine practice, therefore, dextrose, galactose, lactose, levulose, maltose, and saccharose were utilized for preliminary purposes of identification, the reaction with maltose, as Ashford has commented, being the most valuable. These, supplemented with litmus milk and Hiss' semisolid gelatine medium, serve

admirably tentatively to classify the *Monlia* for, characteristically, *Monilia psilosis* shows no reaction in litmus milk and forms the so-called "inverted pine tree" in gelatine stab cultures.

It will be noted from Table 4 that there were usually obtained from the patients other organisms which were very often confusing. The most important of these is *Monilia albicans*, which apparently occurs with some regularity. Morphologically it is very difficult to differentiate these two *Monilia*. In massive cultures after two to three months *Monilia albicans* justifies its nomenclature by appearing a glistening snowy white, while *Monilia psilosis* has a distinctly dirty grayish yellow or brownish appearance and is much less refractive. In addition, the mycelial downgrowth in the medium is usually much more marked in the *M. psilosis* cultures.

Histologically, I have nothing to add to the original description as given by Ashford. The motile body in a pale vacuole is exceedingly characteristic in fresh preparations which, in combination with the large size of the organism and its round rather than oval outline, makes one reasonably certain of its identity.

Following in general the technic which Martinez(14,15) and Michel(16) developed in their work in Porto Rico on the specific fixation of the complement in sprue, a positive result was obtained in the six cases in which the test was performed, and negative results in over ten controls who complained of other chronic intestinal disturbances. By using a *Monilia albicans* antigen further controls were made. The technic and results are here reported.

*Antigen.*—The antigen was obtained by using three-week-old cultures of *Monilia psilosis* grown on Sabouraud's medium, washed off with sterile distilled water. This was emulsified by mechanical shaking for one hour and standardized by the usual blood counting chamber method. Phenol (0.5 per cent) was added as a preservative, after incubation for three days to secure autolysis. This was then heated to 58° C. for one hour and tested for sterility and hæmolytic or anticomplementary action. A similar antigen was prepared from *Monilia albicans* as control for the specificity of the *M. psilosis* cases. The standard used was 1 cubic centimeter.

*Complement.*—Three-tenths cubic centimeter normal pooled guinea pig serum (ten) was used.

*Amboceptor.*—One cubic centimeter of a 5 per cent suspension of washed monkey corpuscles was utilized.

The Wassermann test was performed with two series of tubes; one, containing 0.1 cubic centimeter of the suspected serum, the other 0.2 cubic centimeter of inactivated serum as control for the anticomplementary action. All set-ups were made in duplicate. Parallel series, using 0.7 cubic centimeter of *Monilia psilosis* and *M. albicans* antigen preparation, were run.

The suspected serum, antigen, complement, and salt solution to volume were heated for forty minutes, then the hæmolytic system was added, and the whole was incubated for one hour before the results were read. It will be noted that the *Monilia albicans* antigen gave faintly positive results in two of the sprue cases as well as in one of the nonsuspected cases. This may indicate a tendency toward group specificity, but apparently will offer no difficulty in the matter of differential diagnosis, especially if the serological reaction is performed quantitively with higher dilutions of the serum.

TABLE 4.—*Complete fixation with Monilia antigens.*

| Patient. | Diagnosis. | *Monilia psilosis.* | *Monilia albicans.* |
|---|---|---|---|
| M.S., I | Sprue; anæmia | ++ | +— |
| M.S., II | do | ++ | — |
| M.S., V | do | ++ | +— |
| M.S., VI | do | + | — |
| M.S., VII | do | +— | — |
| M.S., VIII | do | + | — |
| 9432 | Typhoid | — | — |
| 9446 | Tuberculosis | — | — |
| 9454 | Typhoid | — | — |
| 9462 | Bacillary dysentery | — | — |
| 9467 | Typhoid | — | — |
| 9483 | Amœbic dysentery | — | — |
| 9490 | do | — | — |
| MH–17 | Ankylostomiasis | — | + |
| MH–24 | do | — | — |
| 9521 | Epidemic encephalitis | — | — |

The value of the complement-fixation reaction in the diagnosis of obscure intestinal disturbances simulating sprue, such as the chronic dysenteries, should be inestimable from the point of view of the therapeutist, as it offers apparently a truly specific means of diagnosing this relatively large group of cases.

## DISCUSSION

One of the principal objects of this paper has been to present the extraordinary blood picture noted in this group of cases, and to discuss it in relation to our present knowledge of sprue.

If my enthusiasm and interest have carried me a bit outside the originally intended limits of my presentation, I plead for leniency, in as much as the other phases of the work were almost necessary to establish the cases in a recognized clinical place for the purpose of exposition in regard to the blood findings.

In reviewing the literature of sprue I find relatively few references to the blood. In Ashford's monograph in the new Oxford Medicine there is, I think, the strongest statement on record. I quote it in its essentials, as follows:

> The degree and character of the anemia in sprue as in many profound secondary anemias may approach the pernicious type, and * * * laboratory reports may arouse some doubt as to the nature of this anemia, whether it is secondary or essential. * * * The anemia of sprue becomes the dominant note, however, in some cases, * * * and may remain to oppose obstinately a cure.

Castellani and Chalmers (12) say:

> The blood coagulates slowly, and there is always some reduction of the red cells which may fall as low as 1,000,000 per cu. mm. The color index is *low*, and the structure of the cell is normal. The white cells also may be reduced to 2,800 per cu. mm. The ratio of white to red cells is about 1–400 in bad cases. A differential count shows an increase in the mononuclears and eosinophiles.

Brown (11) in 1906 makes a similar observation in reporting the leucocyte counts in twenty-two cases observed by him, in which the average figures showed percentages of 56 for neutrophiles, 20 for lymphocytes, 16 for large mononuclears, and 8 for eosinophiles. He feels that the red cell diminution is due to a toxic destruction of the circulating erythrocytes, similar to that in arsenic poisoning. His statements seem to be the basis of most subsequent comment.

Bahr (9) in 1914 stated that "though present as a general rule, a great degree of anæmia need not presage a fatal termination."

Aside from these few statements, only isolated reports of single cases are found, giving very little or incomplete information—a red count, a hæmoglobin value, a differential count, and so on.

It has been exceedingly unfortunate that practically no autopsy material has been obtained in the group of cases studied, for examination of the bone marrow and spleen might have assisted us in explaining the mechanism of the production of the anæmia. That the *Monilia psilosis* produces a toxin must be recognized, for experimentally we can cause the death of guinea pigs by

the intraperitoneal or even subcutaneous injection of a few cubic centimeters of a saline suspension of the organism. The further experimental data will be reported in a subsequent paper, but it seems appropriate to mention at this time the fact that there seems to be definite evidence of the presence of a toxin in relation to this particular *Monilia,* in contrast to *Monilia albicans* which can be introduced into experimental animals in almost massive doses without the production of symptoms. On the other hand, the actual reproduction of the disease is extremely difficult to accomplish. This may depend in part on our choice of experimental animals, and in part on the element of time which is usually more or less disregarded, in direct contradiction of the facts as the disease appears in man.

The relatively slow development of sprue in man up to a certain point, followed by the very rapid emaciation and anæmia production, speaks strongly for a cumulative action on the part of the infecting agent. Physiologically, there is resistance up to a certain point; but, once that threshold is reached, the resistance is apparently done away with.

The bone marow in these cases gives every indication of complete paralysis or inhibition, as evidenced by the peripheral blood. We find none of the characteristic stimulating effects which a typical secondary anæmia seems to produce. Instead of activity on the part of the red-forming mechanism we find evidence of increased destruction going on and no compensatory hyperplasia of the marrow. The remaining red cells attempt to act defensively, appearing to shrink a trifle and in that way perhaps becoming slightly more resistant to hæmolysis. We should expect to find marked evidence of red-cell fragmentation in the spleen.

It is interesting to note how well the marrow responds to stimulation by transfusion of healthy red cells in the earlier stages, and how little it affects the picture in the later stages. This is of considerable importance from the therapeutic standpoint for, by improving the general condition of the patient through transfusion, the probable chances of vaccine therapy being of benefit are just so much more enhanced.

Thus far in neither guinea pigs nor rabbits have we been able to produce the blood picture seen in human cases; but there is sufficient circumstantial evidence, I think, to blame *Monilia psilosis* for the production of this severe anæmia, for it is constantly present in those cases, and thus far has not been

recovered from other severe anæmias of a frankly secondary type.

The classification of this anæmia, therefore, offers certain difficulties. Strictly speaking, it is secondary to the *Monilia* infection and, therefore, secondary. From the point of view of histopathology, however, the morphological appearance of the cells and the diminution of platelets and of marrow activity exclude it from what is commonly meant by the secondary type of anæmia; and it does not present the morphological changes associated with the primary or essential anæmia that we call pernicious. It must by exclusion, then, belong to that other small group of cases spoken of loosely as aplastic. In this group we find certain hæmolytic agents which are responsible for a number of the cases; among them we can mention ricin, saponin, and even trinitrotoluol, while in a few no exogenous agent can be detected. The latter are the so-called idiopathic cases. Apparently, from purely circumstantial evidence, we can place *Monilia psilosis* in a position comparable to that of some of the aforementioned toxins, and the anæmia that accompanies it in the classification of aplastic anæmia. Whether experimental evidence will bear out this tentative grouping remains to be demonstrated, but it seems a logical disposition to make of these cases until further work settles the question.

## SUMMARY AND CONCLUSIONS

Eight cases of severe anæmia associated with the sprue syndrome are presented, with their laboratory findings.

The isolation of *Monilia psilosis* Ashford, 1914, from these cases is described, which is the first time it has been reported from the Philippine Islands.

The specific nature of the complement fixation in sprue, using a watery extract of *Monilia psilosis* as antigen, is discussed.

The histopathology of the peripheral blood is described and on the basis of the blood picture a tentative classification of this anæmia as aplastic is suggested.

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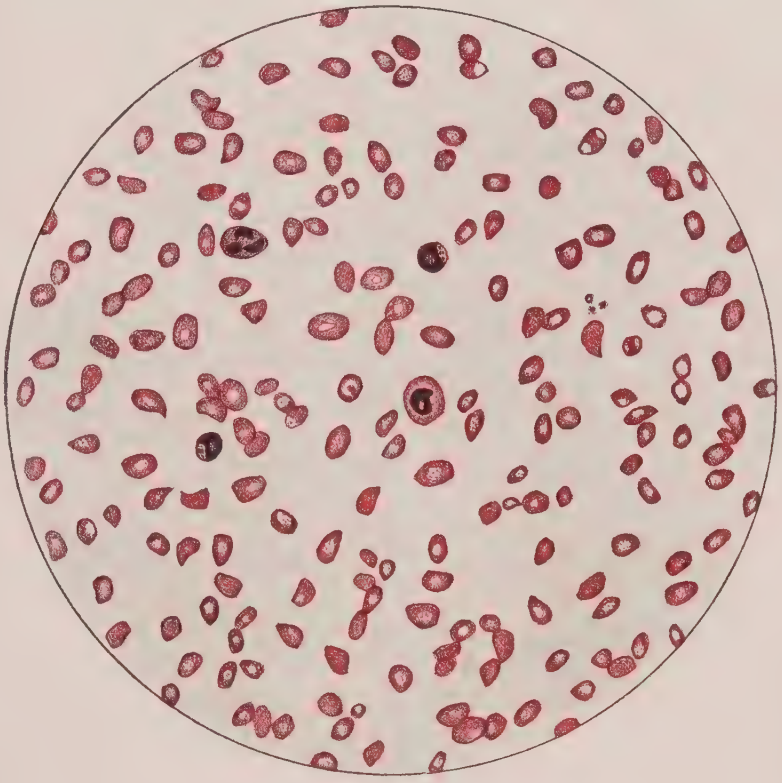
# ILLUSTRATION

PLATE 1

FIG. 1. Blood smear from case M.S., I, sprue, female, 52 years, Spanish. x Zeiss 4 millimeters objective and 12K ocular (15 x). Red blood count, 1,056,000. Relative normal appearance of red cells, only slight anisocytosis and poikilocytosis. Nucleated red cell represents only one seen in twelve examinations. High color index, only slight achromia. White blood count, 2,400. Relative lymphocytosis (approximately 65 per cent of differential count) and small size of lymphocytes. Diminished platelets, 102,000. The blood picture represents typical so-called "aplastic anæmia."

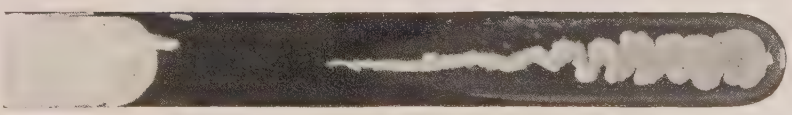
2. *Monilia psilosis*, eight-week-old culture.

3. *Monilia albicans*, twelve-week-old culture.



1

2



3

PLATE 1.

# HYDROIDS OF THE PHILIPPINE ISLANDS

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SIX PLATES

This report embodies the results of a study of the collection of Philippine hydroids made under the direction of Dr. Lawrence E. Griffin, of the University of the Philippines, during 1909, 1912, and 1913, and includes dredgings, scrapings, etc., from the interisland cables from depths of 8 to 177 fathoms.[1] The localities given by Doctor Griffin in his letter accompanying the transmission of the collection are Bantayan Island, Port Galera, Mindoro, Culion, Luzon, Palawan, and Taytay; there were also names on tags found in most of the bottles and jars in which the specimens were preserved. On most of the jars was also a number with the prefix C; for example, C2315, of the precise significance of which I am not advised. Perhaps it relates to a given haul of the dredge or it may be some arbitrary designation. In many cases a record of depth was included, but in none was there any record of latitude or longitude by which a given station could be later located.

Most of the material was preserved in 5 to 10 per cent formalin, but in a few cases alcohol had been used. Owing to the considerable interval before its reception at Syracuse and the unfortunate breakage of several jars in transit, some deterioration had occurred and the condition of perishable portions, such as hydranths and medusa buds, left much to be desired.

The collection comprises some fifty species which were identifiable; nearly half of them are probably new, and others had

[1] Earlier collections of hydroids from Philippine waters had apparently been rather small. The more important reports are: Busk, Voyage of H. M. S. Rattlesnake 1852; Kirchenpauer, Ueber die Hydroidenfamilie Plumularidæ, 1872; Bale, Hydroids, 1882, 1884; Allman, Hydroida dredged by H. M. S. Challenger, Part I (1883), Part II (1888); Marktanner-Turneretscher, Annal. k. k. Naturhist. Hofmuseums Wien (1889–1890); records of Japanese explorations; and still more recently the two admirable monographs by E. Stechow, Hydroidpolypen der Japanischen Ostküste, Part I (1909), Part II (1913). Besides these there are doubtless other records, but the number is relatively small.

been little known. One of the latter, *Idia pristis* Lamouroux, has long been known, but until relatively modern times has been very inadequately described. In this collection it is one of the commonest, but until now I had not seen the species, and at first it seemed like a new find.

The collections were made from one hundred thirty-five recorded stations, but there were doubtless others, as some containers were devoid of record, and from some that were broken in transit the record, if ever present, had been lost. Naturally, many of the records related to the same species, as implied in the fact that the number identified is less than half the total recorded.

Another matter of some interest is the intimate relationship of habitat revealed by this material; that is, many species were apparently always found in what seemed symbiotic relations, a smaller growing upon a larger. This has sometimes been designated as parasitism, but I am inclined to doubt the existence of that relationship among hydroids. In some cases the relations were doubtless merely incidental, as favorable hydroid conditions would be occupied by individuals of two or more species, and their growing together or upon each other is only what all students of hydroids very well know does occur. In the present material this habit was almost universal, an isolated specimen or colony being exceptional.

The study of the collection has been a matter of unusual interest, as it afforded an introduction to a tropical fauna having a large proportion of strange forms, among them the new genus and species *Zanclodea philippina.* This species affords another sidelight upon a problem of long standing and of perplexing difficulty; namely, that associated with Gegenbaur's Cladonemidæ and his genus *Zanclea,* based on the medusa stage alone. Even the medusa has been seldom certainly identified, and the hydroid is unknown to this day.

## Genus **CORDYLOPHORA** Allman

### Cordylophora dubia sp. nov.

Two bottles labeled as from stations C2311 and 2312 contained a few fragmentary specimens, poorly preserved, rendering specific identification difficult. Among those from station C2311, taken in June, 1913, in addition to the *Cordylophora,* were fragments of *Eudendrium, Obelia,* and a sertularian, indicating a marine source. Those from station 2312 were definitely labeled as taken from Mololas River, of the same date as those from

the other. Whether or not there may have been some accidental mixing of the specimens it is impossible to say, though it is not impossible that the *Cordylophora* of the former, true to its variable habit, may have had its habitat in the salt water.

*Trophosome.*—This is distinctively of cordylophoran type, main stem branching alternately, perisarc smooth except at regions of branches, the latter having at their origin a few basal annulations; hydranths clavate rather than spindle-shaped with conical hypostome, the twelve tentacles filiform and tending to assume whorls, though scattered downward upon the hydranth body.

*Gonosome.*—The gonophores were badly preserved and impossible of critical determination as to structure. They usually appeared in clusters or groups on distal parts of branches, ovoid, each borne on its own pedicel; the gonangial capsules were similar to those of *Cordylophora lacustris,* yet in some respects distinctly different. The stems were slenderer, hydranths smaller, and gonangia clearly different.

While the material is such as to render uncertain many aspects of form and structure, and so leaves doubtful any definite pronouncement as to specific independence, it seems well for the purpose of record to list it as a doubtful new species, *Cordylophora dubia,* until better material may furnish final certainty.

## Genus **BOUGAINVILLIA** Lesson

**Bougainvillia philippensis** sp. nov. Plate 1, fig. 1.

Specimens of this apparently new species of *Bougainvillia* were taken at stations 702 and 799, from depths of 40 and 54 fathoms, attached to small bivalve shells, the stolons creeping more or less in the grooves of the shell, and the surface seemingly covered with a mucous mass resembling somewhat that covering the hydrorhiza of *Hydractinia.*

*Trophosome.*—The hydrocaulus grows to a height of 10 to 15 millimeters, stems and branches covered with a smooth, firm perisarc, with occasional indefinite annulations at the origins of branches. Hydranths large, ovoid or broadly spindle form, with conical hypostome from the base of which arise about twenty to thirty filiform tentacles.

*Gonosome.*—Medusæ arise in compact clusters on the upper portions of stem and branches, and in my specimens in various stages of development, from mere buds to almost fully grown medusæ, some with tips of tentacles protruding from the bell, yet it was not possible to determine their structural details. In

shape the body is rather pyriform as seen attached, and the ocelli could be readily distinguished, though the character of the manubrium could not be determined.

So far as I am aware, the only species with clustered medusæ is *Bougainvillia flavida,* described by Hartlaub,[2] though differing in most other features. Bonnevie has also described a species, *B. obscura,*[3] with some features in common, yet very distinct otherwise.

## Genus PERIGONIMUS M. Sars

*Trophosome.*—Hydrocaulus simple or branched; hydrorhiza a filiform creeping or reticular network; hydranths fusiform, with conical hypostome; perisarc rather thin and usually with an outer encrustment of foreign particles extending to the base of the tentacles.

*Gonosome.*—Free medusæ, usually with high bell, and with two to four tentacles (usually two at liberation), arising from large basal bulbs, devoid of ocelli.

### Perigonimus repens Hincks.

*Perigonimus repens* HINCKS, Brit. Hydr. Zooph. (1868) 90; CALKINS, Proc. Bost. Soc. Nat. Hist. (1899) 339.

Among material taken at stations 757, 790, and 879 were hydroids indistinguishable from this species, though owing to poor preservation it was difficult to confirm details of medusoid structures. The material was taken from depths of 10 to 100 fathoms, and mostly adhering to shells and stems of *Tubularia.*

### Perigonimus scandens sp. nov. Plate 1, fig. 2.

The material was taken at stations 738, 778, 861, and 883, at the last from a depth of 110 fathoms. The species differs from any known to me, and from the following description will be recognized as new.

*Trophosome.*—Stems often fascicled in the older portions, and with a generally smooth and transparent perisarc, in striking contrast to that of most known species. Height, 15 to 20 millimeters; branching; hydranths low, vasiform, with domelike hypostome, tentacles rather short, twelve to sixteen; perisarc smooth, annulated at origin of branches.

*Gonosome.*—Medusæ borne on stems and branches, each on its own pedicel, which is annulated; as the medusæ approach maturity the two tentacles become conspicuous and often protrude

[2] Hydromedusen Helgolands (1897) 456–461, pl. 14, figs. 1–10.
[3] Neue norwegische Hydroiden (1898) 7, pl. 1, fig. 4.

from the bell; their basal bulbs are very large and devoid of ocelli.

The species seems to live upon other hydroids, creeping over them in parasitic fashion, and in this material always in such relation. The name proposed for the species is indicative of this climbing habit.

Hartlaub [4] has described a hydroid similar in its trophosome features to that here described, but he offers no specific account, as there were no gonophores present. His species was very minute, 6 millimeters in height and with but eight tentacles, with perisarc extending to their bases, a point of contrast with the above species.

### Genus **CORYDENDRIUM** Van Beneden

**Corydendrium minor** Nutting.

*Corydendrium minor* NUTTING, Bull. U. S. Fish. Comm. Part 3 (1905) 941.

At stations 773, 836, 846, 853, and 855 were taken numerous colonies which seem to be identical with the species described by Nutting from Hawaii. I add such details as seem essential.

*Trophosome.*—Colonies mostly arising from stoloniferous filaments attached most frequently to other hydroids, though occasionally to other objects. I do not recognize any essential aspects of parasitism such as Nutting suggests. Stems average about twice the height given by Nutting, but agree in being fascicled and in most other features. The hydranths are predominantly spindle-shaped rather than pyriform, with about fifteen filamentous tentacles, which are rather promiscuously distributed over the body of the hydranth, with no evidences of whorls.

*Gonosome.*—Medusa buds arise generally from independent branches and singly. As they approach maturity the general organogeny is easily made out. Four radial canals, numerous tentacles folded within the cavity of the bell; manubrium well developed, indicating a period of freedom during the life history; indeed, in handling the specimens they frequently became detached from their pedicels.

### Genus **TUBULARIA** Linnæus

*Trophosome.*—Hydrocaulus with a tubular perisarc, usually unbranched, or irregularly and without annulations. Hydranths

[4] Eped. Antarc. Belg. (1904) 8, fig. 2.

relatively large and flask-shaped, with a basal whorl of filiform tentacles, and a distal or oral series of similar shape, usually fewer in number.

*Gonosome.*—Gonophores medusoid, though not becoming free, borne in racemelike clusters just above the basal tentacles. Eggs develop into actinulæ which are set free and attach themselves as young hydroids.

**Tubularia crocea (Agassiz).**

Specimens of a *Tubularia* taken at stations 846, 849, and 859 were fragmentary and undeveloped, but in all distinguishable respects closely resembled this species. Specimens were small, 30 to 40 millimeters in height, usually with only twelve to sixteen tentacles in each cycle. Gonophores rudimentary. All the specimens were supporting colonies of a symbiotic hydroid, as were many other species. These will be described in another connection.

**Ectopleura dumortieri (Van Beneden).** Plate 1, figs. 3 and 4.

*Tubularia dumortieri* VAN BENEDEN, Rech. sur l'Embryogenie des Tubulaires (1844) 50.

*Ectopleura dumortierii* AGASSIZ, Cont. Nat. Hist. 4: 343; ALLMAN, Ann. & Mag. Nat. Hist. III 13 (1864) 368; HINCKS, Brit. Hydr. Zooph. 1 (1868) 124; BROWNE, Proc. Roy. Soc. Ed. (1905) 748; HARTLAUB, Nord. Plankton (1907) 94; HARGITT, Biol. Bull. 14 (1908) 108; MAYER, Medusae of the World 1 (1910) 69.

This hydroid was taken at station 862, from a depth of 10 to 12 fathoms, in a fine state of development, and laden with medusæ almost ready to be set free. The specimens were somewhat larger than those I have seen before, yet otherwise seem indistinguishable from the above.

*Trophosome.*—Hydrocaulus simple or sparingly branched, about 50 millimeters in height; hydranth large, flask-shaped, with the two series of tentacles of filiform character in general, but the oral series occasionally somewhat capitate. Proximal, about thirty; oral, fifteen to twenty.

*Gonosome.*—Gonophores borne on long, branching pedicels with medusæ in various stages of maturity, the larger with two primary tentacles protruding from the bell, and a second pair visible. None mature enough to show the ectodermal zones of nematocysts.

I cannot agree with the assumption of Mayer that this species is in any specific respect identical with *Ectopleura ochracea.*

My description of the latter was published in 1904, and that of Mayer in 1910 in which he designates it as a synonym. In my description of *E. dumortieri* I designated it as perhaps a variety, namely, *prolifica;* but later comparisons of the descriptions of both Browne and Hartlaub (vide supra), convince me that it is essentially identical with the above, and especially is this sustained by the material here described. Having taken both species frequently at Woods Hole, and compared those with the specimens from the Philippines, I am thoroughly convinced of the validity and distinctness of the two species.

## Genus CORYMORPHA M. Sars

**Corymorpha symmetrica** sp. nov. Plate 2, fig. 5.

The genus *Corymorpha* has generally a northern range, but recently Stechow[5] has described a specimen of this genus, *C. nana,* taken at Plymouth, England.

In the Philippine collection was found at station 2307 a hydroid which is undoubtedly a member of this genus; this goes to confirm Stechow's account just mentioned, and thus again shows that hydroids, like most organisms, may now and then be found far outside their usual range, in the present case very far removed from an Arctic environment.

At first sight it seemed to be fairly similar to *Corymorpha nana,* especially as described by Bonnevie,[6] and is thus especially significant in the light of Stechow's account; but closer scrutiny showed that it belongs elsewhere; indeed, it seems to be distinctly a new species, and also new for this genus from this region.

*Trophosome.*—Hydroid solitary, yet with stoloniferous growths from its base, forming apparently a distinct rhizocaulus, but I found no disposition toward the formation of colonies. The habitat is peculiar; it grows upon masses of a red coralline, which as a background made it very distinct and conspicuous; and in many cases the coral grew about the base of the hydroid. Its characters are distinctively true to type; stems with usual cœnosarcal canals, the peculiar perisarc of the genus, hydranths large and flask-shaped and rather sharply constricted at base; two series of tentacles, a basal series of from thirty to forty which are long and filamentous, depending chiefly on the age of

[5] Journ. Marine Biology 9 (1910–13) 404–406.
[6] Norw. North-Atlantic Exped. (1876–1878) 1899, p. 22.

the specimen, an oral series varying from twenty-four to thirty-six. Hydroid from 20 to 30 millimeters in height.

*Gonosome.*—Medusæ are borne just above the basal tentacles, and on peculiar peduncles, which at first seemed to be an elongate raceme with numerous medusa buds; but, in fact, the latter on my specimens appear to be relatively few, and what at first seemed very small buds were complexly branched masses of nematocysts, as shown in the figures. The largest medusa buds had no apparent asymmetry; no tentacles, but tentacular buds of similar size and position. In shape the medusæ are typical, apparently devoid of functional tentacles, and with faint pigmentlike granules on the outer side; radial and circular canals typical, but no specimen shows the character of manubrium or sex. Largest about 0.7 millimeter in height by 0.6 millimeter in diameter.

The marked symmetry of the medusæ, especially as compared with *Corymorpha nana* (Alder), its nearest relative, distinguishes it from any other known to me.

## Genus EUDENDRIUM Ehrenberg

### Eudendrium attenuatum Allman.

*Eudendrium attenuatum* ALLMAN, Hydroida of the Gulf Stream, Mem. Mus. Comp. Zool. 5 (1877) 6.

Allman's description of this species was based upon the naked stems and branches, neither hydranths nor gonads being present, and he gives it only a provisional name. The material at hand agrees very closely with the description of Allman, and I believe the species to be identical. I therefore add descriptions of the features lacking in the original account, and accept the name given there.

*Trophosome.*—The entire colony is distinctively of the *Eudendrium* type, hydrorhiza filiform and reticulate, stems slender, unfascicled, branching alternately, the branches annulated at origin as are also the pedicels; stem almost devoid of annulation except at its origin from the stolon, or only rarely above; hydranths of usual type, with from twelve to sixteen tentacles, hypostome trumpet-shaped in extension.

*Gonosome.*—The gonads are of the typical sort; females borne in a series on the ends of pedicels having arisen from the hydranth, usually degenerate or entirely aborted; male gonophores lacking in my material.

Taken from a cable at a depth of 100 fathoms, agreeing again in general with Allman's, which was dredged from a depth of 60 fathoms.

Eudendrium griffini Light.

*Eudendrium griffini* LIGHT, Philip. Journ. Sci. § D 8 (1913) 333.

My material was taken at stations 2145 and 2309, in shallow waters, attached to stones and seaweeds. Light's description is extended and renders unnecessary any extended account here.

*Trophosome.*—The hydroid is rather small, from 15 to 25 millimeters, borne on the usual reticulate stolons, stems mostly smooth with occasional annulations; hydranths relatively very large, with numerous (forty to fifty) tentacles, and with trumpet-shaped hypostome.

*Gonosome.*—This is of the usual type, though in the male only about two gonads on a given hydranth, and on opposite sides, with one or two chambers; in females, gonads borne on base of hydranth and of the usual type, though spadix not bifurcated as in *Eudendrium ramosum,* and as they approach maturity the pedicel weakening and falling upon the stem and becoming fused therewith.

This is a very interesting species among the many interesting ones known. *Eudendrium hargitti,* described by Congdon from Bermuda,[1] is hardly less so.

## Genus PENNARIA Goldfuss

Pennaria tiarella McCrady.

*Pennaria tiarella* MCCRADY, Proc. Elliot Soc. Nat. Hist. 1 (1859) 153.

At stations 795 and 2306 colonies of *Pennaria* were taken which in all essential features I recognize as this species, though their state of development is not at that condition which would make the comparison wholly demonstrative, except if one would make such comparison with specimens in similar state. This I have been able to do with material from Beaufort, N. C., and the two show such close correspondence that I have no hesitation as to their identification. No details of description will be offered in this connection, since the species is too well known to call for such. It may be worth while to state that the specimens at station 795 were attached to *Zostera,* a habitat very common with this species, and one that

[1] Proc. Am. Acad. Arts and Sci. 42 (1907) 463.

fortunately corresponds very closely with species of this habitat from Woods Hole.

Pennaria pacifica Clarke.

*Pennaria pacifica* CLARKE, Mem. Mus. Comp. Zool. 35 (1907) 6.

From two stations, 779 and 2310, specimens were taken that are so closely similar in various aspects of structure that I have no hesitation in referring them to Clarke's species. They are rather sharply different as to the trophosome characters, the stems and branches being unlike those of the preceding species in the extent of annulation, the size, and aspects of the hydranth. Clarke [8] has so fully described this species that it is unnecessary to give any details here.

In view of some recently proposed revisions of species of *Pennaria* by Stechow [9] it seems both pertinent and rather imperative to enter some protest and correct certain obvious errors and misinterpretations of fact.

There need be no doubt of the fact that, in those earlier days when the taxonomist reckoned his success by the number of species described, the temptation to multiply species was responsible for much confusion; but, on the other hand, any hasty reaction and unwarranted nullification of long-standing and well-founded species must inevitably lead to further confusion. I was among the first to show the importance of giving critical attention to the influence of varied environmental conditions upon such plastic organisms as hydroids, in an early paper on the development of *Pennaria tiarella.*[10] On the basis of this study Clarke soon after took occasion to correct certain errors of his own (vide supra). Stechow's attempt to reduce all known species of *Pennaria* to a very few, or perhaps one, seems too radical and reactionary for serious consideration.

I have studied with much care the development of several species of *Pennaria,* and have compared the morphology of both the hydroid and the medusoid aspects of species from widely separated geographic and hence climatic regions, such as Australia, the Mediterranean, Philippine Islands, Porto Rico, Bermuda, Tortugas, etc. I believe that, while there are cases in which species have been founded on local variations, such as *P. gibbosa* and *P. tiarella,* which I sought to correct,[11] for the

[8] Mem. Mus. Comp. Zool. 35 (1907) 6.
[9] Zool. Jahrb. 32: 336.
[10] Bull. U. S. Bur. Fisheries 24 (1904) 32.
[11] Loc. cit.

most part the species earlier established are based upon good characters. The contention of Stechow that temperature may be a serious disturbing feature in its relation to species may be well worthy of consideration, but his application of this to *Pennaria* is a direct reversal of the facts involved. For example, his attempt to interpret the liberation and free life of the medusæ of *Pennaria* as being due to the low temperature of deeper waters, while the fact that those with a habitat on eelgrass during midsummer do not liberate the medusæ is attributable to the higher temperature which lessens vitality, is the very opposite of what actually happens. In *P. tiarella* those liberating the medusæ in great numbers are the summer variety, while those which mature the medusæ in early summer and from deeper waters never liberate them. It is thus in *P. australis* also. During midsummer medusæ are liberated in enormous numbers and, as in *P. tiarella,* during early evening. In the case of *P. cavolini* the medusæ seem never to be set free. At two different times I have worked at the Naples laboratory during the height of the breeding season and, in spite of every effort to secure free medusæ of the species, I never succeeded in observing or taking them in the tow. Doctor LoBianca, an expert on the fauna of the Neapolitan waters, assured me that medusæ of *Pennaria* are never set free from the hydroid. It seems safe, therefore, to say that conditions of food or temperature can have little direct influence upon the very important point under review.

In *Pennaria cavolini* the distinctions are sharper than in any other species at hand. The pedicels are constantly short, averaging about 0.6 millimeter, and are constantly and completely annulated. The hydranths are shorter than in the other species, and the medusæ fixed.

In *Pennaria australis* the general aspects of the hydroid are similar to those of the preceding, pedicels short, averaging from 0.6 to 0.7 millimeter, but with only about five annulations at the base; medusæ constantly free, and larger than in any of the other species.

In *Pennaria pacifica,* while the general features are similar to those of *P. tiarella,* again the pedicels are shorter, averaging from 1.4 to 1.6 millimeters, and with five or six annulations at the base.

In *Pennaria tiarella* there is a marked difference in these respects; pedicels slender and from 3 to 3.75 millimeters, with five or six annulations at the base.

## CLADONEMIDÆ Gegenbaur, 1856

### Genus **ZANCLOIDEA** novum

*Trophosome.*—Hydrocaulus well developed, branching, and with dense brownish perisarc; hydranths clavate, with conical or rounded hypostome; tentacles of two sorts, filiform, irregularly distributed over the body, in many cases heavily annulated and bearing numerous masses of nematocysts; a second series, few in number, orally situated, and capitate.

*Gonosome.*—Gonophores medusoid, with bell well developed, four radial canals, two marginal tentacles, provided with abaxial tuffs of nematocysts borne on stalks; exumbrella free of meridional rows of nematocysts; no ocelli.

Type, *Zancloidea philippina* sp. nov.

**Zancloidea philippina** sp. nov. Plate 2, fig. 6.

At a number of stations, among them 764, 850, 871, 879, and 883, were taken colonies of a hydroid of very unusual characters and most puzzling as to its systematic relations. Finally specimens were discovered which bore nearly mature medusæ whose characters at once showed affinities with the Cladonemidæ. Careful staining and mounting demonstrated in a few cases the protruding tentacles from the medusa, with the stalked nematocysts of *Zanclea* and *Gemmaria.* At first it was thought that in this hydroid we might have at last the long-sought hydroid stock of Gegenbauer's *Zanclea costata;* but the medusæ showed the presence of only two tentacles and, moreover, absence of the exumbrellar rows of nematocysts. Again, the hydroid characters differed in most respects from those of *Gemmaria,* the only genus in which the hydroid is really known.

*Trophosome.*—Hydrocaulus stout, 30 to 50 millimeters in height, irregularly branched, with dense brownish perisarc, generally smooth surface, but with a lamellalike structure, and with annulations only occasionally near base of stems or origin of branches. The interior of the perisarc occasionally shows indications of annular thickenings, similar to those figured by Hincks in *Zanclea implexa.* Hydranths very large, 3 to 5 millimeters in length by about 0.5 to 0.7 millimeter in diameter in larger regions; shape clavate, becoming larger toward the oral region; hypostome rounded, with capacious mouth. In one case two distinct hypostomes and mouths were present. Tentacles numerous and irregularly disposed over the entire body

of the hydranth, chiefly filiform, with occasionally the appearance of a few capitate ones about the mouth; most of them definitely characterized by strong annulations composed chiefly of massed batteries of nematocysts, especially on the distal portions.

The characters of the hydroid will be seen to differ so markedly from those of the hydroids of *Gemmaria* as to exclude it from this genus; but the same is true when one tries to find any clearer affinities with other genera. As will be noted, the medusoid characters point strongly to the Cladonemidæ.

*Gonosome.*—The medusæ are borne over the entire body of the hydranth, each apparently on a single pedicel, though it is common to find the buds arising in clusters, and in some cases they seem to have a common basal peduncle, as I have shown in the case of *Gemmaria implexa* and as is also true of *G. gemmosa.* Some of the medusæ seemed to be approaching maturity, the tentacles being protruded from the bell, and in these cases showed clearly the stalked nematocysts mentioned above. Largest buds 0.5 millimeter high, by about half that in width. Color pinkish in preserved material, and in one case a note gave that as the color of fresh specimens.

While there can hardly be reasonable doubt as to the affinities of the species with the Cladonemidæ, it is likewise even less doubtful that we have to do with an undescribed species. This is particularly true of the hydroid whose distinctive characters differentiate it from any species or genus known to me. Except for the medusan characters, which are strongly suggestive of the cladonemids, it would be difficult to place it under any existing hydroid family. The characters of the hydranth have little indicative of any gemmarian affinities, and the facts that *Zanclea* was established solely on medusan characters, its hydroid being absolutely unknown to this time, and that *Z. implexa* has been adjudged by highest authority to belong to *Gemmaria* leave the genus *Zanclea* without a single clearly recognized hydroid stock. In making this statement I do not overlook the contention of Hartlaub [12] who would identify *G. implexa,* given by me in 1904, as a different species which he designates as *Z. hargitti,* basing the distinction upon the single question of the medusæ being distributed over the hydranth body and frequently several upon a single peduncle as contrasted with some earlier descriptions which designate them as

[12] Nord. Plankton 12 (1907) 115, 119.

borne upon a single pedicel and in a whorl below the tentacles—a contention altogether trivial in the face of facts familiar to anyone who has studied the colonies in the height of their breeding season. Having in an earlier paper [13] submitted a brief account of some facts related to this point, I do not deem it necessary to go into further details here.

Mayer [14] has discussed certain phases of this problem, and assigns a specimen of *Gemmaria gemmosa* [15] to *Zanclea costata* Gegenbaur, provisionally but wholly gratuitously. There are also several errors of fact in the account; for example, designation of McCrady's medusa as having four tentacles, and as being 6 millimeters in height by 4 millimeters in width—points absolutely unmentioned by McCrady. Further, it has been observed repeatedly that this species at Woods Hole comes to productivity with only two tentacles and with a maximum height of 1.7 millimeters.

Bigelow [16] describes these medusæ as having a maximum diameter of 1 millimeter, and having but two tentacles, but with ripe gonads, which indicates that they have attained full size. I may merely point out that while Bigelow refers to my earlier discussion of Hartlaub's views he curiously ascribes to me full agreement therewith, the very point against which I had strongly protested.

It remains to refer to a still later account bearing on our problem [17] which describes a medusa designated as *Zanclea implexa* which has four tentacles but differs in two other points from the species as known; namely, the lack of the exumbrellar perradial nettle rows, and the presence of well-defined abaxial, purple-red ocelli on the tentacle bulbs, characters absolutely unknown in any species described. In spite of these facts Neppi ascribes it without hesitation to the species above named, explaining the absence of the perradial nettle rows as probably attributable to their degeneration as the medusæ approximate maturity! He also gives the depth from which it was taken as 1,000 meters, a further fact hard to conceive of in a species distinctively of surface habitat!

[13] Biol. Bull. 14 (1908) 100–106.
[14] Medusae of the World (1910) 87.
[15] Bull. Mus. Comp. Zool. 37 (1900) 35.
[16] Mem. Mus. Comp. Zool. (1909) 187–188.
[17] Neppi, Valeria, Adriatische Hydromedusen, Kais. Akad. Wiss. Wien 121 (October, 1912) 11.

Even among the best taxonomists the problems of these sections of Cladonemidæ are extremely perplexing. This I believe to be due largely to the unfortunate disregard of first principles of scientific taxonomy; namely, complete knowledge of the entire life cycle. This was long ago pointed out by such masters as Agassiz and Allman, the former giving expression to the idea in the following words, referring directly to hydroids: [18]

> A true regard for science ought to lead us to imitate the entomologists, who raise the larvæ of insects before naming them.

Only two years later the latter states:[19]

> It will assuredly seem strange that those principles of classification which have been acknowledged as the only sound ones, and which have been our guide in every other group of the animal kingdom, should be almost entirely ignored in our attempt at a systematic arrangement of the Hydroids.

**Cladocoryne floccosa Rotch.**

*Cladocoryne floccosa* ROTCH, Ann. & Mag. Nat. Hist. IV 7 (March, 1871) 228; ALLMAN, The Gymnoblastic Hydroids, London (1871) 380; DU PLESSIS, G., Mitt. Zool. Sta., Naples 2 (1881) 178; HARGITT, Biol. Bull. 17 (1909) 369.

Material collected at station 746 from a cable at a depth of 25 fathoms contained, along with other hydroids, a few colonies of this beautiful hydroid. The species agrees admirably with the descriptions of the above-named authors, the last excepted, and is further considered in a later section.

*Trophosome.*—Stems simple, from a reticular hydrorhiza, and averaging about 7 millimeters in height; hydranth prominent, with long and branched tentacles from its base, ten to twelve in number, and terminating in knobbed masses of nematocysts; a second whorl of about six simple tentacles about the mouth, ending in similar knobs.

The entire trophosome is well protected with a perisarc, extending to the base of the flask-shaped hydranth; it is mostly smooth, or with occasional annulations.

This material agrees in all essentials, including depth, habitat, etc., with that described by du Plessis (vide supra), but seems to lack the batteries of nematocysts which both he

[18] Natural History of the United States 4 (1862) 339.
[19] Ann. Nat. Hist. (May, 1864) 345.

and Rotch describe as located between the tentacle bases on the body of the hydranth. I find no evidence of these in my material.

*Gonosome.*—This is absent in my material, but du Plessis describes it very fully in his extended paper just referred to.

In this connection I desire to correct certain points of my former account (vide supra), wherein I described a form that I found on *Sargassum* at Woods Hole in 1909 and designated as a varietal form of *Cladocoryne floccosa,* which form I named *sargassensis,* from its habitat on this seaweed. At that time I was not aware of a species described by Allman [20] and named *C. pelagica* from its floating habitat. As I stated at the time, the species differed in several respects from *C. floccosa;* but, comparing it now with this material and the description of Allman, and that later of Inaba cited by Stechow,[21] I am convinced that the Woods Hole species is identical with *C. pelagica,* and take this opportunity so to designate it.

Clytia delicatula (Thornely).

*Clytia* sp. INABA (1890) figs. 34, 35.
*Obelia delicatula* THORNELY, Zool. Results 4 (1900) 453.
*Campanularia delicatula* JADERHOLM, Neue oder wenig bekannte Ostasiatische Hydroida (1902).
*Clytia delicatula* STECHOW, Hydroidpolypen der japanischen Ostküste Part II (1913) 65.

Specimens of my material were taken at station 2130, and agree in all essentials with the descriptions of the above-named authors. The confusion as to genus by both Thornely and Jaderholm was probably due to inadequate material, or misinterpretation of the nature of the gonosome. It remained for Stechow finally to clear up the case, using freely the description of Inaba.

My material was collected by Light in April, 1913, at Taytay, Palawan, and was growing upon barnacles (*Lepas*), which were attached to a piece of bamboo that had been washed ashore.

Clytia kincaidi (Nutting).

*Campanularia kincaidi* NUTTING, Hydroids from Alaska and Puget Sound (1899) 743.
*Clytia kincaidi* FRASER, Hydroids of Vancouver (1914) 146.

From stations 732 and 743, among other hydroids, were specimens which I believe to be of this species, though absence

[20] Journ. Linn. Soc. 12: 251.
[21] Hydroidpolypen der japanischen Ostküste, Part II (1913) 50.

of gonosomes makes impossible an exact identification. In my material it was not easy to distinguish the deeply fluted hydrothecæ described by the above-named authors, yet in all other respects they agree.

**Clytia alternata** sp. nov. Plate 2, fig. 7.

Material from stations 2310 and 2313, Port Galera, Mindoro, among other hydroids, contained a species which appears to be new.

*Trophosome.*—Hydrorhiza a creeping reticulum, stems rising to a height of 10 to 20 millimeters, sparingly branched, with hydrothecæ arising alternately, giving to the stem a somewhat geniculate aspect, and borne on long annulated pedicels, campanulate in shape, margins with about twelve very acute teeth, the distal portions of the walls very delicate and often appearing to be fluted; hydranths large, with about fifteen tentacles and rather conical hypostome, but poor preservation makes this point doutbful.

*Gonosome.*—Gonangia elongate, obconical and with distal portion abruptly truncate, borne in axils of the stem and hydranth, on short annulated pedicels; orifice smooth with slightly everted lips; medusæ borne in a single row along the blastostyle.

The species resembles somewhat *Clytia linearis,* but careful comparison only emphasizes the distinctness.

**Clytia tubitheca** sp. nov. Plate 2, fig. 8.

From station 765 was found a species of *Clytia* rather unique in the greatly elongated hydrothecæ, averaging about 0.6 millimeter in length by 0.2 millimeter in diameter. Gonothecæ, 0.5 millimeter in length by about 0.25 millimeter in diameter. Pedicels annulated, the entire length of stems being about 1 millimeter. Margins of hydrothecæ dentate with faint striæ parallel therewith, the entire theca being cylindrical, with a short constriction at the base to articulate with the pedicel.

*Gonosome.*—Sessile, elongate, rather clavate, and abruptly truncate, orifice smooth. Medusæ present in various stages of development.

This is the only species of *Clytia* known to me with distinctly cylindrical hydrothecæ, though Agassiz has described one, *C. cylindrica,* which is claimed to have such, but most of the published figures fail to show this.

A species described by Marktanner-Turneretscher has some resemblance to this one, but its trophosome shows distinct dif-

ferences, and it is also doubtful in that no gonosomes are present.[22]

**Clytia longitheca** sp. nov. Plate 3, fig. 9.

The material containing this hydroid was taken at stations 732 and 874. On first study it seemed to conform in its trophosome character with Allman's *Obelia longicyatha,*[23] which was based upon the trophosome alone. It was later referred by Pictet to *Clytia,* but I have not seen his description, and depend upon those of Billard[24] and of Nutting[25] but have some doubt as to the identification after study of the present material. Except for the marginal teeth, which are single in Nutting's species, it compares well with that here described.

*Trophosome.*—The stems arise from a creeping stolon, somewhat reticular, at first simple but soon branching profusely, and often becoming fascicled by the downgrowth of tubes from the base of branches. Further reference is made to this in the description of *Obelia longitheca* below. Height of stems, 20 to 50 millimeters, hydrothecæ long, obconic, distal portions very delicate and hyaline, and margins with about ten pairs of acute teeth, becoming somewhat fluted parallel with the teeth; pedicels short and annulated, hydranth large, with about twenty long stout tentacles.

*Gonosome.*—Gonangia very long and club-shaped, medusæ in all aspects of development, some in process of escape from the gonangium.

The species is one of the largest known to me, and is very beautiful.

**Obelia longitheca** sp. nov. Plate 3, fig. 10.

Numerous colonies of an *Obelia* that seems to be new were taken at stations 690, 855, 864, 878, and 884. Except in size it has much in common with a hydroid described by Thornely as *Gonothyrea longicyatha.*[26] Her description was confirmed by Stechow in material obtained at the entrance of Uraga Kanal, Japan, 1904.[27] The absence of gonangia in his material, and what may have been a misinterpretation of the gonosome by Thornely, led both to assign the hydroid to *Gonothyrea.* I

[22] Die Hydroiden des k. k. Naturhist. Hofmuseums Wien (1889) 215.
[23] Mem. Mus. Comp. Zool. 5 (1877) 10.
[24] Exped. Scientifique du Travail. et Talisman 8 (1907) 168.
[25] American Hydroids, Pt. III (1915) 58.
[26] Willey, Zool. Results, Pt. III (1900) 451.
[27] Hydroidpolypen der japanischen Ostküste, Part II (1913) 71.

strongly suspect that it should have been assigned to *Obelia*, an impression that is strengthened by an admission of Thornely as follows:

> If what appears to be an external capsule is in reality an escaping medusiform zooid, the species may be an *Obelia*.

*Trophosome.*—Hydrocaulus large, 8 to 12 centimeters high, branching profusely, stems fascicled in older parts, and showing downgrowths of tubular structures similar to those described by the above-named authors for their *Gonothyrea*. This is not, however, a character of any specific note, since it is common in many species. Hydranths large and long, with about fifteen filamentous tentacles arising below the hypostome, which is trumpet-shaped but much longer than usual, as will be seen from the figures. Hydrothecæ very long and with about ten pairs of acute teeth about the margin, pedicels rather long and variably annulated, in many cases totally so, while in others they may comprise only the basal and distal parts. The size is also variable, especially in material from different stations. For example, those from station 690 were 0.9 millimeter long, pedicels averaging 0.35 millimeter, with annulation variable; in those from station 855, hydrothecæ averaged 1.18 millimeters long, pedicels about 0.5 millimeter; those from station 878 averaged about 0.9 millimeter long, pedicels about as in the others.

*Gonosome.*—Gonangia long, slender, and club-shaped, averaging 1 millimeter long by 0.25 millimeter in greater diameter; medusæ were present in the gonangia in large numbers and in all stages of development, some in process of escape with tentacles, about thirty in number, extended.

Allman has described an *Obelia longicyatha*,[28] but his account dealt with the trophosome only and the transfer of the hydroid by Pictet (see under *Clytia longitheca*); it has many points of likeness with the one here recorded but, for obvious reasons of distinction and to avoid confusion, I have proposed it as a new species under the above caption.

Obelia sp.?

An unrecognized species of *Obelia* was taken at station 737, at 24 fathoms, which seems worth while recording, though absence of the gonosome makes an attempt to determine its specific relations little more than guesswork. The trophosome

[28] Mem. Mus. Comp. Zool. 5 (1877) 10.

is characteristically Obelian, stems from a filamentous rhizocaulus branching and with hydranths arising in axils, pedicels short, annulated, and with large hydrothecæ with undulating, or obscurely toothed, hydranth with about twenty filamentous tentacles. Gonosome absent.

Obelia attenuata sp. nov. Plate 3, fig. 11.

At station 765, associated with several other hydroids, was found a species of *Obelia* apparently undescribed. In the general shape of its trophosome it resembles *Obelia longitheca*, yet in most other respects its characters are very different.

*Trophosome.*—Hydrocaulus simple or sparingly branched, very slender, about 15 millimeters high, hydrothecæ arising alternately, elongate obconic, margins with about ten pairs of bimucronate teeth like those of *Obelia longitheca*, 0.5 millimeter long, on short annulated pedicels. Base of stems occasionally fascicled.

*Gonosome.*—Gonangia elongate, clavate, ending abruptly, 0.55 millimeter in length by 0.2 millimeter in average diameter. Genital products apparently medusoid, but too young to afford positive characters, borne on a central blastostyle.

The above name is proposed with some doubt, and is given provisionally as a basis of record.

Silicularia rosea Meyen. Plate 4, fig. 12.

*Silicularia rosea* MEYEN, Ueber d. Leuchten d. Meeres (1834) 204.
*Hypanthea aggregata* ALLMAN, Hydroida of the Challenger Expedition, Pt. II (1888) 26.
*Silicularia rosea* JADERHOLM, Hydroiden aus Antarktischen und Subantarktischen Meeren (1905) 17; NUTTING, American Hydroids, Pt. III (1915) 91.

Colonies of this hydroid were taken at stations 857 and 867, all of them small but in good condition, though without gonangia; taken from a cable at depths of 60 and 150 fathoms. Stems simple, from creeping stolons, base of stems expanded into enlarged supporting portion and conical at the point of extension of the very slender and amber-colored stems or pedicels, so thickened as to obscure the inner cœnosarc. Hydrothecæ rather hemispherical, with greatly thickened walls and oblique margins, in many cases with a fissurelike expression of the lower obliquity of the cup. Entire height from 2 to 5 millimeters.

According to Nutting, "This genus is found only in the Southern Hemisphere, and most of the species are in the subantarc-

tic region." On this basis the one here recorded will materially extend its distribution to the Northern Hemisphere.

Nutting makes Allman's *Hypanthea aggregata* synonymous with the species under consideration, but a comparison of their figures with my own will show considerable differences. I have some doubt as to their identity, yet hesitate to propose a new specific name, especially in the absence of the gonosome.

**Silicularia minima** sp. nov.

At station 2311 there was taken, with small colonies of *Obelia* and *Perigonimus*, an extremely small specimen of an undescribed *Silicularia*. Like the former species it has a symbiotic habitat on these hydroids. Like the former, also, the trophosome seems to comprise a stoloniferous, climbing mass from which the single peduncles arise, each with its hydranth. These are very minute, the former varying from 0.3 to 0.4 millimeter in height, hydranths and tentacles from 0.1 to 0.2 millimeter in length; the hydrotheca is rather shallow, bilateral, but attached to the peduncle direct, thus lacking the spherical annulus of the former species.

While there may be some uncertainty in giving to the species a distinctive name, in the absence of gonophores, still, with the distinctness of the hydranths and the extreme minuteness of the specimens, there can hardly arise confusion should later discovery fail to confirm its distinctiveness, and as a basis of record it seems desirable to designate it by the name here given.

## Genus **HEBELLA** Allman

*Generic characters.*—Hydrocaulus a creeping, monosiphonic stolon. Hydrothecæ cylindrical, with entire margin, destitute of operculum, and with cavity differentiated from that of the peduncle.

Gonosome unknown.

**Hebella corrugata** (Thornely).

*Campanularia corrugata* THORNELY, Ceylon Pearl Oyster Fisheries & Marine Biology, Part II (1904) 114.

*Hebella corrugata* VANHOFFEN, Die Hydroiden der Deutschen Südpolar-Expedition (1910) 314; STECHOW, Hydroidpolypen der japanischen Ostküste, Part II (1913) 105, 107.

This hydroid was very common among the material in this collection, taken at stations 793, 824, 839, 844, 860, and 893, and doubtless would have been noted in others also had critical search been made for it. It was always in association with

other hydroids, perhaps parasitic. The stolons creep over the stems and branches, often in a complex way, but predominantly in a direction parallel to the stem or branch to which they are attached. Hydrothecæ very large, often curved, with corrugated walls and everted margins, and often reduplications; pedicels short and obliquely annulated. Hydranths rather elongate, with conical hypostome, and with about twelve tentacles. In my specimens hydranths always retracted. Gonosome unknown.

The species was first described by Thornely as *Campanularia corrugata,* and later identified as a *Hebella* by Vanhoffen. I have followed chiefly the account of Stechow as cited above.

**Hebella contorta** Marktanner-Turneretscher.

*Hebella contorta* MARKTANNER-TURNERETSCHER, Die Hydroiden des k. k. Naturhist. Hofmuseums (1889) 215, figs. 17*a* and *b*.

A species of *Hebella* taken repeatedly, associated with *Idia pristis,* and which I take to be identical with the one named above and described by its author from material reported from Singapore, also associated with *Idia pristis,* and especially recorded from stations 778 and 824. Hydrothecæ long, cylindrical, with everted margins, often contorted variously, which condition I was at first inclined to regard as due to mechanical distortion made during examination and, especially, by the technic of staining and mounting but which was later found to be due to some natural cause during the growth of the hydroid. Hydrothecæ 0.65 millimeter long by 0.2 millimeter in diameter, pedicels very short and plain; hydranths long and slender, with conical hypostome and about ten tentacles. The gonosome is unknown.

**Filellum serratum** (Clarke).

*Laforea serrata* CLARKE, Bull. Mus. Comp. Zool. 5 (1879) 243; BILLARD, Exp. Scient. du Travail. et du Talisman 8 (1907) 178.
*Filellum serratum* STECHOW, Hydroidpolypen der japanischen Ostküste, Pt. II (1913) 111, 112, fig. 85.

Like the preceding species, this was always associated with other hydroids, presumably as symbionts, and in most respects is conformable with the descriptions given by the above-named authors. The hydrothecæ are adnate for about half their length to the stolons, this portion being marked with rather definite serrations giving the appearance of sawteeth, probably due to the wrinkling of the tubes in those regions. The gonosome is unknown.

Genus **HALECIUM** Oken, 1815

**Halecium lighti** sp. nov. Plate 4, fig. 13.

This species was taken by Prof. S. F. Light at Port Galera Bay, Mindoro, attached to tubes of *Eunice* growing in the strong currents flowing in and out of the bay. In another jar were also specimens, numbered 2315, Galera Bay, attached to a coral clump, in strong current, evidently in the same locality, also collected by Light.

Light had recognized the fact that the hydroid was a new species and had in his description proposed for it the specific name *armatum*, based upon the presence in many hydranths of a pair of extra large tentacles, some of which seemed to be armed with especially large nematocysts; hence the name. Describing the hydranths he had stated "each hydranth bearing on opposite sides two shortened and thickened, curved, club-shaped tentacles (nematodactyls) armed along either side with a row of from 9 to 15 large nematocysts." This particular detail of his description proved to be only partly true, large numbers of hydranths being entirely devoid of these specialized tentacles, some having but one, and thus rendering the specific designation proposed very doubtful and even misleading. Light's description was, by his own option, turned over to me when the collection was assigned to me for investigation and complete report, with the suggestion that I use such part of his description as I found acceptable. This I am glad to do, and in acknowledgment of his courtesy in the matter I take pleasure in naming the species in his honor as indicated above. I am also taking advantage of certain of his details of description in the account of the species.

*Trophosome.*—Stems about 25 millimeters in height, fascicled in basal portions, branches regular, and both stem and branches divided by straight joints into internodes of approximately the same length; branches and hydrophores in same plane, the latter adnate for most of their length, and in many cases where extension or reduplication occurs extending outward with slightly everted margins. The series of punctæ, so characteristic a feature of many species of *Halecium,* are lacking or extremely obscure in this one. Light gives the dimensions of the hydrophores as about those of the stem of internode on which found; diameter of base of internodes at base of branch, about 0.12 millimeter; of stem, 0.15; length of internodes of stem, about 0.4; of branches, about 0.3.

*Hydranths.*—In general these are fairly comparable with those of many other species; long and nonretractile, resting on an expanded basal portion by which they are attached to the hydrophores. The shape of the hydranths is somewhat distinctive; the figures will give a fairly good impression of this feature. As will be noted, two rather marked constrictions occur, one just below the circle of tentacles, another just below the expansion upon which it is attached to the hydrophore where it is linked to the cœnosarc of stem or branch. The median region is beautifully spindle-formed, the whole comprising a graceful body including hypostome, tentacles of which the number varies from twenty-five to thirty-five; neck, body, base, and its attachment to the hydrophore as already noted. The color is white, both in life and in its preserved state, unless discolored by the preservative.

*Gonosome.*—In this species as yet wholly unknown.

The following discussion of the distribution of *Halecium* is that given by Light in the description referred to above:

> *Halecium* has a very wide bipolar distribution, being found in the colder and temperate waters of all seas. With this there are only three species known from the tropical seas and these all from the tropical Pacific. There is no doubt however that a more careful study of the hydroid fauna of the Tropics will reveal the presence of several more. Of the 57 described species of the genus, some of which are no doubt synonyms, the originals came from the following regions: Arctic and north temperate 39, distributed as follows: Alaska 6, Pacific coast of the United States and Puget Sound 6, Atlantic coast of the United States and Gulf Stream 6, Bermuda 2, North Atlantic 2, North Sea 1, Norway 2, British Isles 11, Adriatic 1, Antarctic and South Temperate including Southern Australia 14; Tropical Pacific 3.

While these do not comprise all known species, as to distribution they none the less show in a general way the scope of the *Halecium* fauna of the seas as known.

**Idia pristis** Lamouroux. Plate 4, fig. 14.

> *Idia pristis* LAMOUROUX, Hist. des Polyp. Coral Flex. Zoophytes (1816) 200 (English translation); BALE, Australian Hydroid Zoophytes (1884) 113, Further Notes on Australian Hydroids (1893) 104; ALLMAN, Hydroida of the Challenger Exped., Pt. II (1888) 83, pl. 39.

Specimens of this remarkable hydroid were taken at many stations, 685, 718, 744, 824, 839, 860, 2314, and others. While long known, as shown in the old account of Lamouroux, it has not been generally known. Specimens of the present collec-

tion are the first seen by me, and records of its distribution are rather few in the literature available. Bale reports it from some five localities about the coasts of Australia; Allman, from two localities of the Challenger account, Panay, P. I., and off Bahia; and Marktanner-Turneretscher, Philippines. In the present collection it is one of the commoner species. Its bathymetric range is from 15 to 30 fathoms as recorded by the above-named authorities.

*Trophosome.*—Stems erect, stout, attaining a height of 75 to 130 millimeters, though doutbless more; pinnæ alternate, rather regular, and divided into segments fairly well marked, but this feature is not very evident on the main stem, where it is often obscure or lacking. Allman's descriptions seem too regular and precise to apply to my specimens, as I have had occasion to point out in other connections, and I can verify the statement of Bale that his specimens of gonothecæ "differ considerably from specimens" described by Allman, whose figures seem to have been given for the sake of their picture value rather than as precise representations of actual structure.

*Gonosome.*—The gonangia are borne chiefly but not solely on the stem, as I have found many occurring on pinnæ as well. The figure given is an accurate sketch of a female gonangium; that of the male is slenderer and slightly longer and somewhat less deeply fluted than in the female. In this distinction I am giving what appear to be male and female organs, but the material is not such as to afford an actual demonstration. My specimens fail to show the puncta emphasized by Allman, but not described by others. On the neck of the gonangium are frequently found small particles adhering to the capsular surface, but they are not at all comparable with the puncta of *Halecium.* Again, my specimens show transverse wrinkling of the capsules, as figured by Busk and as given by Bale in his account. Moreover, in my specimens the longitudinal flutings are less numerous than as given by Allman, averaging about ten, often less and rarely over twelve, while he gives sixteen in his figures.

## Genus **STEGAPOMA** Levinson

**Stegapoma medusiformis** sp. nov. Plate 4, fig. 15.

*Campanularia fastigiata* ALDER, Ann. & Mag. Nat. Hist. III 5 (February, 1860) 73.

*Calycella fastigiata* HINCKS, A History of British Hydroid Zoophytes (1868) 208.

*Stegapoma gracilis* NUTTING, Hydroids of the Hawaiian Islands, Bull. U. S. Fish. Comm. (1905) 944.

At stations 854 and 866 were found several colonies of this very interesting and beautiful hydroid, the first of its kind to come under my direct observation. They were taken from a depth of 110 fathoms, and in every case were attached to other hydroids, parasitic (?) in relationship.

*Trophosome.*—Made up of creeping stolons, simple pedicels, and hydrothecæ, growing over stems of *Obelia,* usually standing out at a right angle from the supporting hydroid. The pedicels are slender, about as long as the hydrotheca, which measures in large specimens about 1.6 millimeters in length by 0.32 millimeter in diameter in the middle region; operculum made up of a series of strips which meet in a rooflike ridge at the top of the theca. Hydranths large and elongated, with conical hypostome and about twelve filiform tentacles, apparently supported by a diaphragm as shown in the figure, though it was difficult to demonstrate.

*Gonosome.*—The gonangia are long and club-shaped, sessile from the stolon, and 3.5 to 4.2 millimeters long by about 0.6 millimeter in median diameter. Blastostyle lateral and bearing medusæ in various stages of development. So far as I am aware, these have not been described heretofore. In my specimens they show their distinctive medusoid characters, as fig. 15 will show, and sections made in both long and short directions confirm the surface aspects. These sections fail to show the presence of germ cells at this stage, which implies that the medusæ are liberated and lead a free life for a time and that the sex cells are set free during this free-living stage. The form of the medusa is fairly typical, bell rather high and narrow, with gastric structures of usual form and structure, with few tentacles; only two show in specimens within the gonangia. I regret the preservation was not such as to afford good microscopic structures, but these were sufficient for the study of the general morphology.

In general the species here described agrees very well with those referred to above, but it agrees more closely with that of Nutting, *Stegapoma gracilis*; yet there appear features which differ from it. I am disposed to suggest that on the basis of the distinctive medusoid characters, and the larger size of the gonangia, it be designated as a new species.

## Genus THUIARIA Fleming

**Thuiaria tubuliformis** (Marktanner-Turneretscher). Plate 4, fig. 16.

*Dynamena tubuliformis* MARKTANNER-TURNERETSCHER, Die Hydroiden des k. k. Naturhist. Hofmuseums Wien (1890) 70.
*Thuiaria tubuliformis* NUTTING, American Hydroids, Pt. II (1904) 70.

The material was taken at several stations, among them 761, 793, and 817. The colonies were mostly small and none more than 40 to 50 millimeters in height. Trophosome made up of a reticulate mass of stolons from which stems grew erect, but with a definitely geniculate aspect from the alternate origin of the branches; this was particularly apparent in younger specimens and distally; hydrothecæ tubular and deeply immersed in the stem, less so on branches, the distal portion facing outward, the aperture closed by two valves which meet at the two opposite teeth of the theca. In my specimens the hydrothecæ are arranged in pairs, varying in number for each internode from two to four or five, giving the branches a distinctly segmented aspect, resembling somewhat *Pasythea,* a fact not shown in Nutting's figures nor mentioned in his descriptions. In fact, my specimens agree more closely with those described by Marktanner-Turneretscher than with Nutting's account. I regret that no gonangia were found in my collection, nor does the earlier author describe them. Nutting's figures are difficult to understand, since he gives three drawings of the gonangia, all different; in two the orifice has straight margins, in the other the margin is very much everted. Again, his figures of the stems and branches show such great difference that they might almost be considered as from different species, a fact which perhaps may illustrate how inadequate are trophosome characters in many cases as a basis of specific determination.

**Thuiaria quadrilateralis** sp. nov. Plate 5, fig. 17.

At station 736 was taken a species of *Thuiaria* which does not seem to have been described; at any rate, I am unable to identify it with any known species.

*Trophosome.*—Colony rather stout, stems arise from a reticulate series of stolons, to a height of 30 to 50 millimeters with branches alternating from successive internodes, each of which bears three hydranths, the pinnæ arising just below the third hydranth of the internode; hydrothecæ tubular and deeply sub-

merged in stem and branch, with apparently valves attached to inner and outer margins.

*Gonosome.*—Gonangia large, several times the size of hydrothecæ, of four-sided shape, borne on short pedicels of pinnæ, none on stems of my specimens. The quadrilateral aspect of these organs is rather unusual, and so far unknown among the *Thuiaria.* The specific name is proposed from this character.

Genus **SERTULARIA** Linnæus (in part)

**Sertularia minuta** sp. nov. Plate 5, fig. 18.

This hydroid was taken at station 697 along with fragments of several other hydroids. It is a very minute species, yet apparently mature, as there are numerous gonangia.

*Trophosome.*—The stems are simple, with no signs of branching in any specimen; they arise from creeping stolons, which also bear the gonangia. Height of colony, 4 to 7 millimeters, each bearing from four to six paired hydrothecæ on the upper portion of the stem, which is regularly divided into internodes each with a single pair, adnate for about a third of their length, tubular, the distal part strongly divergent and with apparent abcauline valve; though the delicacy of these terminal portions makes difficult an exact demonstration, in some cases two valves seemed present. Hydranths long and very slender, but preservation was not such as to make structural features demonstrable.

*Gonosome.*—Gonangia arise from the stolons, though close to the base of a stem, in some cases seeming to attach to the stem, but this was doubtful; the shape is broadly spindlelike, and deeply and regularly corrugated, as shown in the figure, with rather large aperture, from which in some specimens embryos were in process of liberation.

This species has some points of resemblance with *Sertularia pourtalesi,* but in size and shape of hydrothecæ there are strong differences, especially as described by Allman and Nutting, the figures of Marktanner-Turneretscher being much nearer my species.

**Sertularia dubia** sp. nov. Plate 5, fig. 19.

This minute hydroid was taken at station 2308, Port Galera, Mindoro. No gonangia were present, and the specimens are apparently young, but I am not able to identify the species with any known to me; so, for purposes of record, I propose the name *dubia* to distinguish it.

The stems are simple, composed of regular internodes, each with a single pair of hydrothecæ which are relatively large, and with correspondingly large hydranths, each with about fifteen to twenty tentacles. The hydrothecæ have their distal portions strongly divergent, as in the previous species, and with a two-valved operculum.

**Sertularia sigmagonangia** sp. nov. Plate 5, fig. 20.

The colonies of a hydroid, apparently new, were taken at stations 704 and 2310, from a cable at the north shore of Batan, at depths of 15 to 177 fathoms.

*Trophosome.*—Stems erect, rigid, with branches at right angles of stem and also rigid and ungraceful; color brown, perisarc dense; hydrothecæ of stem alternate, those of branches strictly opposite, rather retort-shaped, and two opercular valves not easily distinguishable; hydrothecæ face directly outward, but those of stem just below origin of branch recurve downward in many cases.

A point of some importance is the rather constant reduplication of the margins of hydrothecæ, which does not appear in *Sertularia versluysi,* the species most nearly resembling it.

*Gonosome.*—These organs are elongate and fusiform, but rather sigmoid in general outline, due apparently to the direct outgrowth, then an upgrowth, and finally a curving of the distal portion with orifice facing directly outward, the margins ending in some four inconspicuous teeth, the whole gonangium deeply corrugated and some three or four times the length of the hydrotheca, 1.7 and 0.6 millimeter in length and diameter at broadest part, respectively.

I believe the species to be new and propose for it the name suggested by the shape of the gonangium.

## Genus **SERTULARELLA** Gray

**Sertularella gayi**? Plate 5, fig. 21.

At station 768 were taken fragmentary colonies of a hydroid resembling somewhat *Sertularia annulata* of Allman,[29] yet differing in some respects. The stems are straggling, irregularly branched, and indefinitely divided into internodes; hydrothecæ alternate, usually two to each internode, divergent from stem or branch and narrowing to four-angled margin, with four teeth and a four-valved operculum, distal and upper part of

[29] Hydroida of the Challenger Exped. (1880) 52.

thecæ corrugated. No gonangia on my specimens. In some aspects it resembles *S. gayi*?; yet, as stated above, with strongly marked differences. I merely list it for record under this name.

Sertularella philippensis sp. nov. Plate 6, fig. 22.

At stations 729, 788, and 882 were taken hydroids of this species, the best and most typical coming from 882.

*Trophosome.*—Stems from reticular stolons attached to rocky fragments, dense and stout, yellowish brown, profusely branched and in alternating order, as are the hydrothecæ of both stem and branches, divided into fairly regular internodes by indistinct nodes; hydrothecæ rather short and broad with apparently four-valved openings, the valves difficult to demonstrate; hydranths short, with some fifteen tentacles, body in expansion round, but in contraction folded or retortlike.

*Gonosome.*—Gonangia are large, 2.5 millimeters long by about 0.8 millimeter in diameter, and with broad aperture and three strong and prominent marginal teeth.

Sertularella punctagonangia sp. nov. Plate 6, fig. 23.

At station 884, from a depth of from 65 to 150 fathoms, along with large masses of *Obelia longitheca,* there were taken specimens of a sertularian which seemed quite new, and careful scrutiny of available literature confirmed this impression. The hydroid is a beautiful one, both as to form and structure, as will be noted in the description and figure given.

*Trophosome.*—The specimens were from 50 to 75 millimeters in height, stems slender, amber color, more or less erect and with alternate branches, also delicate and relatively long and curving gracefully, the ends attenuate. Hydranths of stem alternate, a single one occupying the axil of each branch, but those of the branches more or less opposite in position, all of delicate hyaline aspect, and facing outward, tubular and attached for about a third of their length to stem or branch, then curving to a narrowed orifice with what appears to be a single abcauline valve. Hydranths elongate, slender, and attenuate, with conical hypostome, and fifteen to eighteen, delicate, long, and threadlike tentacles. Stems and branches irregularly annulated, but not showing definite divisions into segments.

*Gonosome.*—The gonangia are numerous, relatively large and with smooth transparent walls, and borne on stems, occasionally

on branches, usually attached just below a hydrotheca. They are elongate, urnlike structures, with slightly everted margins, each with a convex lidlike operculum, hinged at one point. The beauty of shape is heightened by a series of glistening puncta, variable in number, around the outer shallow constriction, as shown in the figure. So far as I am aware, this is the only instance of such puncta in the Sertularidæ. In these specimens the gonangia were apparently male.

As will be noted, the description given of the hydroid shows characters not entirely peculiar to this genus, such as both alternate and nearly opposite positions of the hydrothecæ on stems and branches, respectively, and the distinctly unusual aspects of the gonangia. Since it does not appear to be better allied elsewhere, it is tentatively placed here as designated above.

## Genus **SYNTHECIUM** Allman

The following is Allman's characterization of the genus which he established in 1888: Trophosome, stem divided into regular internodes, each of which carries a pair of opposite hydrothecæ, or a single one which alternates with those of the adjacent internodes; hydrothecæ adnate for about half their length with the mouth facing outward and with margins everted and often reduplicated.

*Gonosome.*—Gonangia borne on peduncles which spring from the interior of cavity of certain hydrothecæ where they replace the hydranths.

**Synthecium flabellum** sp. nov. Plate 6, fig. 24.

Several colonies of this hydroid were taken at station 715, Mindanao. The specimens were mostly small, some having a height of 30 to 40 millimeters, but others not more than 15 millimeters. Branching is usually opposite and in the same plane, and frequently terminates in elongate filaments, much as in *Diphasia* and many other hydroids; hydrothecæ opposite and adnate for about half their length, margins smooth and mostly reduplicated. In my specimens the hydranths were badly preserved and the characters not determinable.

*Gonosome.*—Gonangia were borne on both stem and branches, and in these specimens mostly female, ova being recognizable in a looplike spadix not unlike those of *Eudendrium ramosum*, the whole inclosed in the gonangium which is spindlelike but rather depressed and podlike. In certain specimens which ap-

peared to be male, though too poorly preserved to enable one to determine it, there was less of flattening and the generative mass was centrally located.

In these specimens I am unable to confirm certain of the features emphasized by Allman. For example, I often find branching to be alternate, or only a single branch appearing on a given internode, and this is very usual with secondary branches. Again, I find that these branches may arise just as in other hydroids, but also in a telescopic aspect as mentioned by Nutting in *Synthecium rectum.*[30] I also find similar variation as to the hydrothecæ, usually opposite and regular, but frequently single and more or less irregular. I find the same variation as to gonangia. In the main they are as described in the original generic account, but not infrequently they arise without the appearance of the hydrothecal envelope, just as with a branch. I am quite inclined to agree with the contention of Torrey[31] that the origin of the gonangia here is not the unique feature Allman has claimed.

## Genus **PASYTHEA** Lamouroux (in part)

**Pasythea griffini** sp. nov. Plate 6, fig. 25.

At stations 771 and 794 were taken colonies of a hydroid of an unknown species. Its generic characters were unmistakable, and except in size its general appearance was similar to *Pasythea nodosa* or *P. quadridentata;* but there were sharp differences, the most convincing being the gonangia which were unlike any known.

*Trophosome.*—Stems mostly simple, but occasionally branching, as noted in *Pasythea nodosa;* borne on creeping stolons which on eelgrass often made a loose reticulum, but those on mollusks did not show this; height of stems from 4 to 9 millimeters, and as in other species, the lower segment frequently with a single pair of hydrothecæ, the upper ones with from two to four pairs. It differs from other species known to me in that the nodes are not oblique but square, forming usually a joint resembling an annulus of the form very characteristic of hydroid stems, and the internodes are very short, differing sharply in this character from other

[30] American Hydroids, Pt. II (1904) 135.

[31] The Hydroida of the Pacific Coast of North America, Univ. of Cal. Publ. Zool. 1 (1902) 62.

species. Another feature was rather interesting as showing its possible mode of differentiation to the typical sertularian type; namely, that rarely the stem may have but a single pair of hydrothecæ on each internode.

*Gonosome.*—The gonangia of this species are very distinctive. They usually arise from the basal internode, but they also arise from other internodes; occasionally three were noted on the basal node, those above usually single; the shape is rather urn-like, with a very short pedicel, outline smooth, and with an elongate, rather narrow neck curved outward and with beautifully everted margin, as shown in the figure.

A species, described from the Philippines by Marktanner-Turneretscher and probably quite distinct though no gonangia were present, is similar in size to *P. griffini,* but they have few characters in common.

In this connection, I may direct attention to the fact that, since the original description of *Pasythea nodosa,*[32] I have carefully compared that material with material of later date, which is identical with the original descriptions of *P. quadridentata,* and find the distinctive trophosome differences very well marked. The later identification of material by Stechow [33] with *P. nodosa* still further confirms the species, allowing for possible modifications which may come from discovery of the gonosomes.

## Genus **PLUMULARIA** Lamarck (in part)

**Plumularia ramsayi** Bale.

*Plumularia ramsayi* BALE, Australian Hydroid Zoophytes (1884) 131.

From station 2311, in June, 1913, Light collected a few fragments of several hydroids, chiefly *Idia pristis,* a *Eudendrium,* an *Obelia,* and small colonies of the above-named hydroid which, though fragmentary, were fairly well preserved. The colonies were devoid of gonangia. They were, however, sufficiently distinct to reveal fairly definite specific characters, which made possible the recognition of the close relationship of this hydroid to Bale's species, though they differed in several points; for example, in the branching which is very regular in my specimens and alternate in others. On this point Bale states that there is "no regularity in their arrangement, as they may be either opposite or alternate, and there are often two or three branches on one side to one on the other." However, the specimens at

[32] Biol. Bull. 14 (1908) 114.

[33] Hydroidpolypen der japanischen Ostküste, Part II (1913) 150.

hand are too few and fragmentary to warrant contention, and as Bale's description designates the species as "extremely variable in habit," it seems better to accept his account than to attempt a detailed description from the material in my possession.

Plumularia sp.?

There was another, very small fragment from Taytay, Palawan, of a *Plumularia* of doubtful specific relation, though very distinct from the preceding one, and altogether too fragmentary to warrant any attempt at specific determination. It seems to be very young, rising to only 4 to 6 millimeters in height, branching dichotomously, and supported from a filamentous stolon.

The accounts here, and that of Busk describing *Plumularia effusa*, listed by Bale,[34] seem to be the only records of *Plumularia* from the Philippines.

## Genus **ANTENNELLA** Allman

**Antennella gracilis** Allman.

*Antennella gracilis* ALLMAN, Mem. Mus. Comp. Zool. 5 (1877) 38; NUTTING, American Hydroids, Pt. I (1900) 77.

At stations 749 and 798 colonies of the above-named hydroid were obtained from the submarine cables near Calbayog, at depths of 49 to 54 fathoms. In all essentials except size the specimens agree well with the descriptions of Allman. My specimens averaged only about 12 millimeters in height, and unfortunately were devoid of gonangia, which thus far are unknown. Following Allman, Nutting regards the species as stemless, that which appears to be stem being considered a hydrocladium arising direct from the stolons. It is not clear that this view has any particular value. Why not as well designate it as a case of variation, or perhaps better, mutation, or even regression, or other expression of adaptation, as Hincks has very strongly suggested concerning *Plumularia secondaria*?[35]

## Genus **SCHIZOTRICHA** Allman

**Schizotricha philippina** sp. nov. Plate 6, fig. 26.

A very delicate and beautiful hydroid was taken at station 839, but no data as to depth or other particulars were recorded.

[34] Op. cit. p. 130.

[35] A History of British Hydroid Zoophytes (1868) 304.

*Trophosome.*—The hydroid is a very delicate one, about 25 millimeters in height, the stem arising from reticular stolons; the main stem has annular segments in the basal part, and above is divided into fairly regular internodes, the joints being both square and oblique; hydrocladia sparingly branched, usually only once; hydrothecæ cuplike, one to each internode, and with a nematophore just below and a pair attached to the base of the theca.

*Gonosome.*—The gonangia are curved cornucopialike, resembling those of *Schizotricha tenella,* but with relatively broader openings, attached by very short pedicels to the hydrocladium just below the base of the hydrothecæ; walls are very delicate and collapse under the slightest pressure, and are apparently devoid of nematophores.

So far as known to me, this is the first record of a member of this genus from the Philippine Islands.

## Genus **DIPHASIA** Agassiz

**Diphasia digitalis** Bale.

*Desmoscyphus longitheca* ALLMAN, Mem. Mus. Comp. Zool. 5 (1877) 26.

*Diphasia digitalis* BALE, Australian Hydroid Zoophytes (1884) 101; NUTTING, American Hydroids, Pt. I (1900) 110.

At stations 781 and 893 were taken sterile colonies of a species which is in all essentials identical with that above named, at least so far as trophosome characters are concerned. The colonies were more or less fragmentary, attached to fragments of coral rock, seaweed, etc. They were apparently small, about 25 millimeters in largest specimens, while Nutting gives the height as 4 inches. The branching was not regular, with no secondary branches such as figured by Allman. The presence of long terminal growths or filaments was strongly marked in some cases, which is a well-known character of other species also.

## Genus **THYROSCYPHUS** Allman

**Thyroscyphus simplex** Allman.

*Thyroscyphus simplex* ALLMAN, Hydroida of the Challenger Exped., Pt. II (1888) 25.

At stations 769, 792, and 893 were taken colonies of a hydroid which I identify as *Thyroscyphus simplex,* though no gonangia were present; indeed, none have been described, so far as I am aware. Several features in my material differ

from the descriptions and figures of Allman. In several instances I have had occasion to criticize the mechanical aspects of his figures, and in this species his figures differ very much from my specimens. For example, the stems are often devoid of distinguishable segmentation; again, one set of specimens, those from station 769, had hydrothecæ of very different form from that of specimens from the other stations named above. In other respects they were quite alike; so I interpret the widely divergent hydrothecæ in these as an individual peculiarity attributable to conditions of growth or environment, and would also so interpret the differences of nodes, the excessive thickenings of the stems or branches having more or less obscured the joints.

**Aglaophenia macgillivrayi** (Busk).

*Plumularia macgillivrayi* BUSK, Voyage of the Rattlesnake (1852) 400.
*Aglaophenia macgillivrayi* ALLMAN, Voyage of the Challenger, Zool., Pt. I (1883) 34; MARKTANNER-TURNERETSCHER, Die Hydroiden des k. k. Naturhist. Hofmuseums Wien (1889) 268.

From stations 873, 897, and 898 several fine colonies of this rather remarkable hydroid were taken from the reef near Mindanao, at a depth of 8 to 10 fathoms. The species was first described by Busk as *Plumularia macgillivrayi;* the next account is that of Allman, in which he describes in much detail a very large specimen, "upward of fifteen inches high." My specimens averaged about 20 centimeters.

*Trophosome.*—Colony massive, stem thick, fascicled, with numerous opposite branches, all devoid of hydrothecæ; secondary pinnæ and hydrocladia, the latter with rather deep hydrothecæ having smooth margins; median and lateral nematophores, the median adnate to the hydrotheca and not extending beyond its margin, the lateral rather broad and extending beyond the margin of the hydrotheca. An interesting feature of some of these specimens was the presence of stolonlike outgrowths from upper and terminal portions of stems and branches, especially where some evidence of injury was apparent.

*Gonosome.*—Corbulæ similar to those of other species, borne on primary or secondary pinnæ, but never on the stems. In these specimens there was no distinguishing evidence of sex.

The extended description and excellent figures of Allman cited above render unnecessary fuller details here.

## Genus **LYTOCARPUS** Kirchenpauer

### Lytocarpus philippinus Kirchenpauer.

*Lytocarpus philippinus* KIRCHENPAUER, Ueber die Hydroiden der Familie Plumularidæ (1888); MARKTANNER-TURNERETSCHER, Hydroiden des k. k. Naturhist. Hofmuseums Wien (1889) 274; NUTTING, American Hydroids, Pt. I (1900) 122.

From station 803 were taken hydroids of a species which seems identical with that named above, though there are several points of difference, as will be noted.

*Trophosome.*—Stems coarse, fascicled in lower portions, and with a height of 8 to 15 centimeters, ascending from a knotted rootlike mass of fibers. Colony branching alternately, with pinnate hydrocladia directed forward and upward; hydrothecæ arranged rather closely, each with a deep constriction anteriorly, median and lateral nematophores of the usual form but differing somewhat from Nutting's description, the median ones extending beyond the hydrothecal margin, while the lateral ones do not extend much if any beyond it.

*Gonosome.*—The gonangia are ovoid, flattened laterally, the capsular walls rather thick but transparent; usually a single gonangium borne on the greatly reduced phylactocarp, but occasionally two. No distinction as to sex was apparent.

While the differences named are not great, they involve both gonosome and trophosome. Still I am inclined to regard them as not of sufficient importance to warrant any definite pronouncement of more than varietal values.

### Lytocarpus sp. ?

At station 896, from a cable at a depth of 45 to 54 fathoms, near Samar, colonies of a large hydroid were found which apparently belongs to this genus, though absence of gonangia makes it difficult to be certain, the trophosomal characters being very similar in this genus and in *Aglaophenia.*

*Trophosome.*—Colony large, ascending from a mass of rootlike fibers, with a height of about 30 centimeters. Stems fascicled, devoid of nematophores or hydrothecæ, branches alternate, and with beautiful pinnate hydrocladia with an average length of about 10 millimeters. The hydrothecæ are rather deep and cuplike, with undulating or crenular margin, the anterior having a spinelike tooth.

Gonosome lacking.

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# ILLUSTRATIONS

[All figures, except 4 and 14, are magnified about 24 diameters.]

PLATE 1

FIG. 1. *Bougainvillia philippensis* sp. nov.
2. *Perigonimus scandens* sp. nov.
3. *Ectopleura dumortieri* (Van Beneden).
4. *Ectopleura dumortieri*, portion of gonophore, highly magnified.

PLATE 2

FIG. 5. *Corymorpha symmetrica* sp. nov.
6. *Zancloidea philippina* sp. nov.
7. *Clytia alternata* sp. nov.
8. *Clytia tubitheca* sp. nov.

PLATE 3

FIG. 9. *Clytia longitheca* sp. nov.
10. *Obelia longitheca* sp. nov.
11. *Obelia attenuata* sp. nov.
12. *Silicularia rosea* Meyen.

PLATE 4

FIG. 13. *Halecium lighti* sp. nov.
14. *Idia pristis* Lamouroux, gonangium, magnified.
15. *Stegapoma medusiformis* sp. nov.
16. *Thuiaria tubuliformis* (Marktanner-Turneretscher).

PLATE 5

FIG. 17. *Thuiaria quadrilateralis* sp. nov.
18. *Sertularia minuta* sp. nov.
19. *Sertularia dubia* sp. nov.
20. *Sertularia sigmagonangia* sp. nov.
21. *Sertularella gayi* ?

PLATE 6

FIG. 22. *Sertularella philippensis* sp. nov.
23. *Sertularella punctagonangia* sp. nov.
24. *Synthecium flabellum* sp. nov.
25. *Pasythea griffini* sp. nov.
26. *Schizotricha philippina* sp. nov.

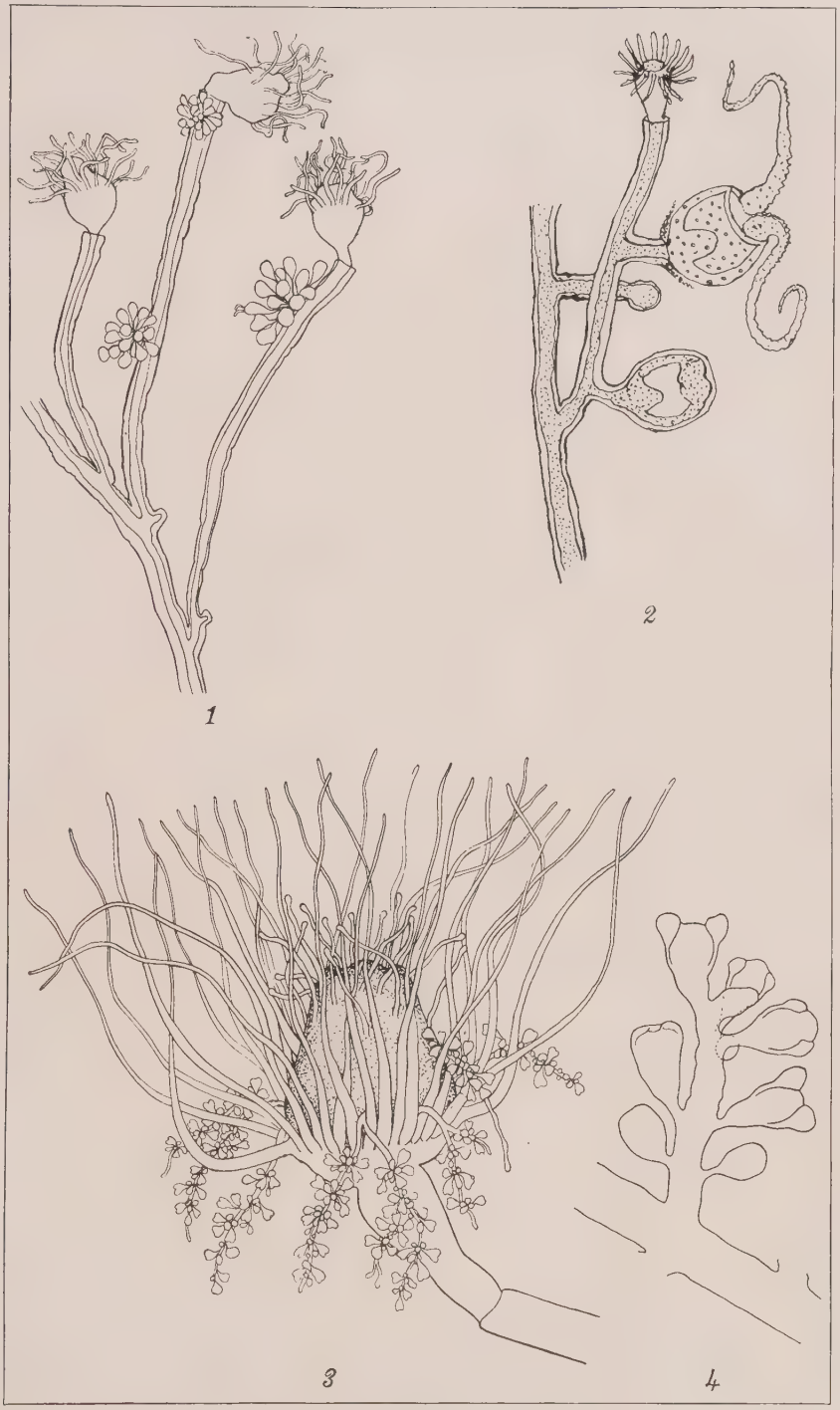


PLATE 1.

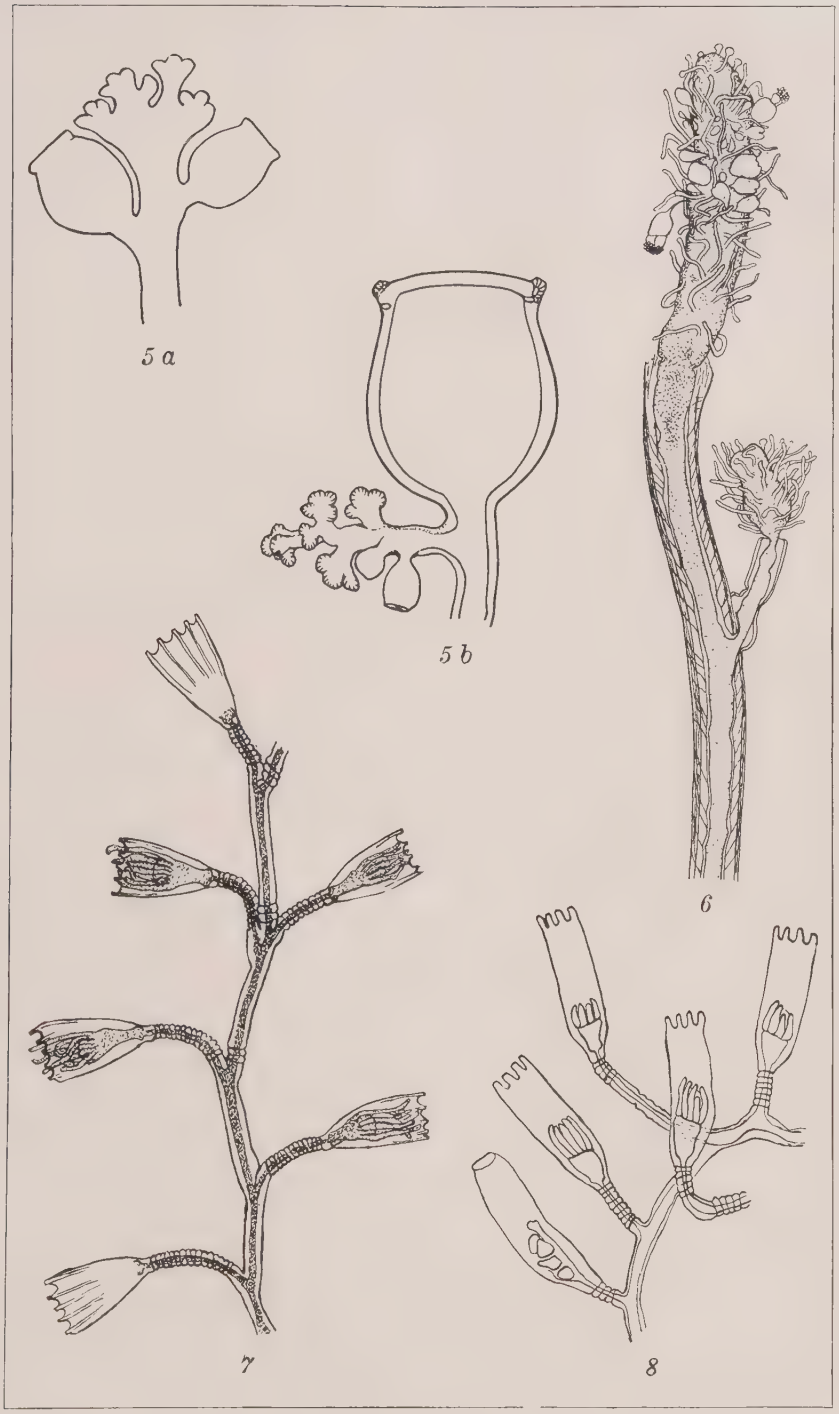


PLATE 2.

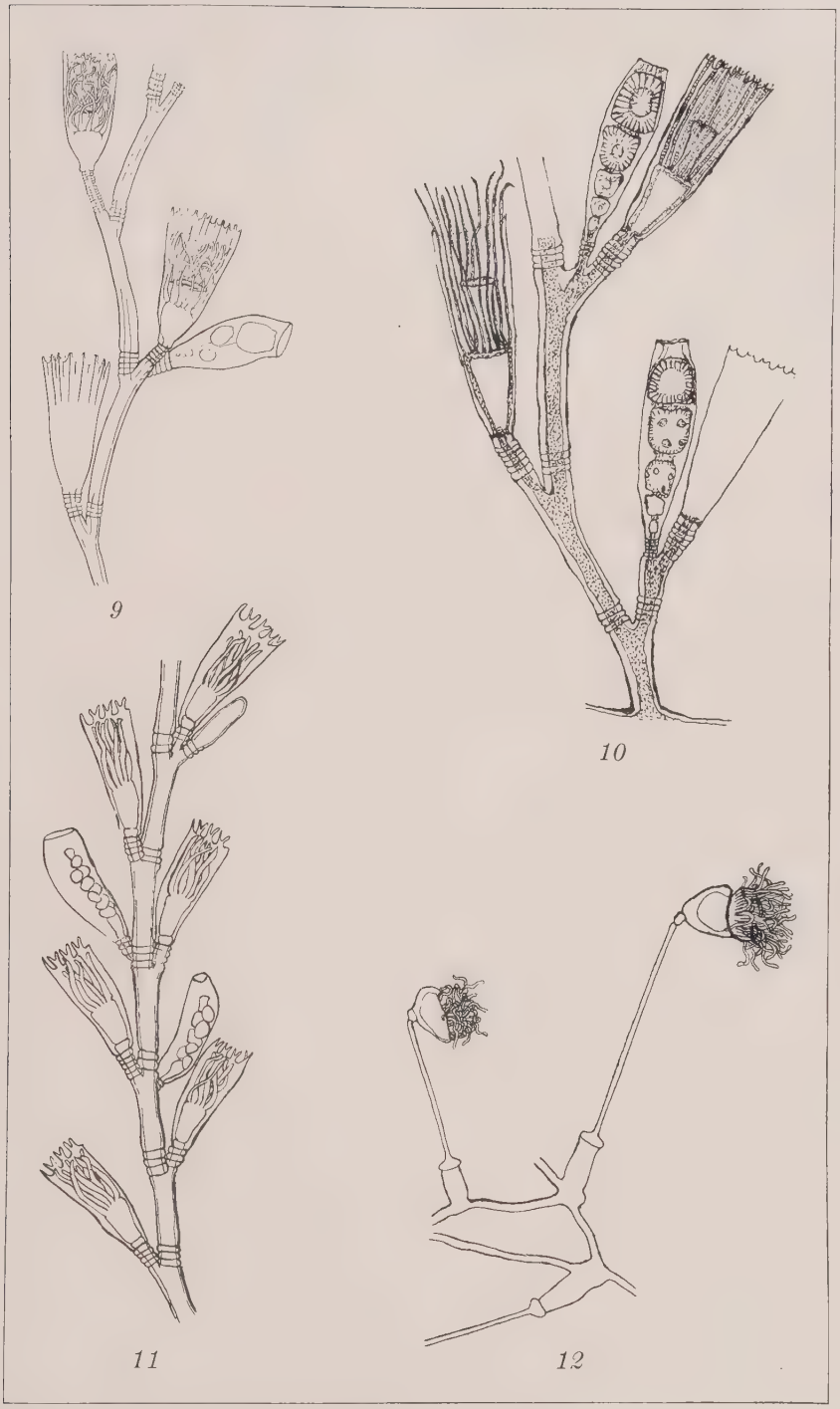


PLATE 3.

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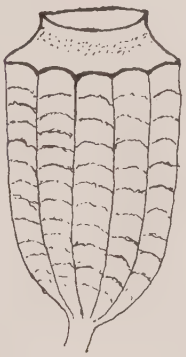

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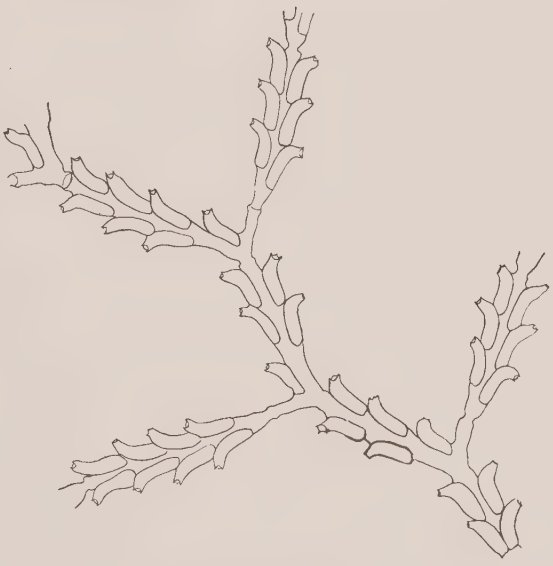

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PLATE 4.

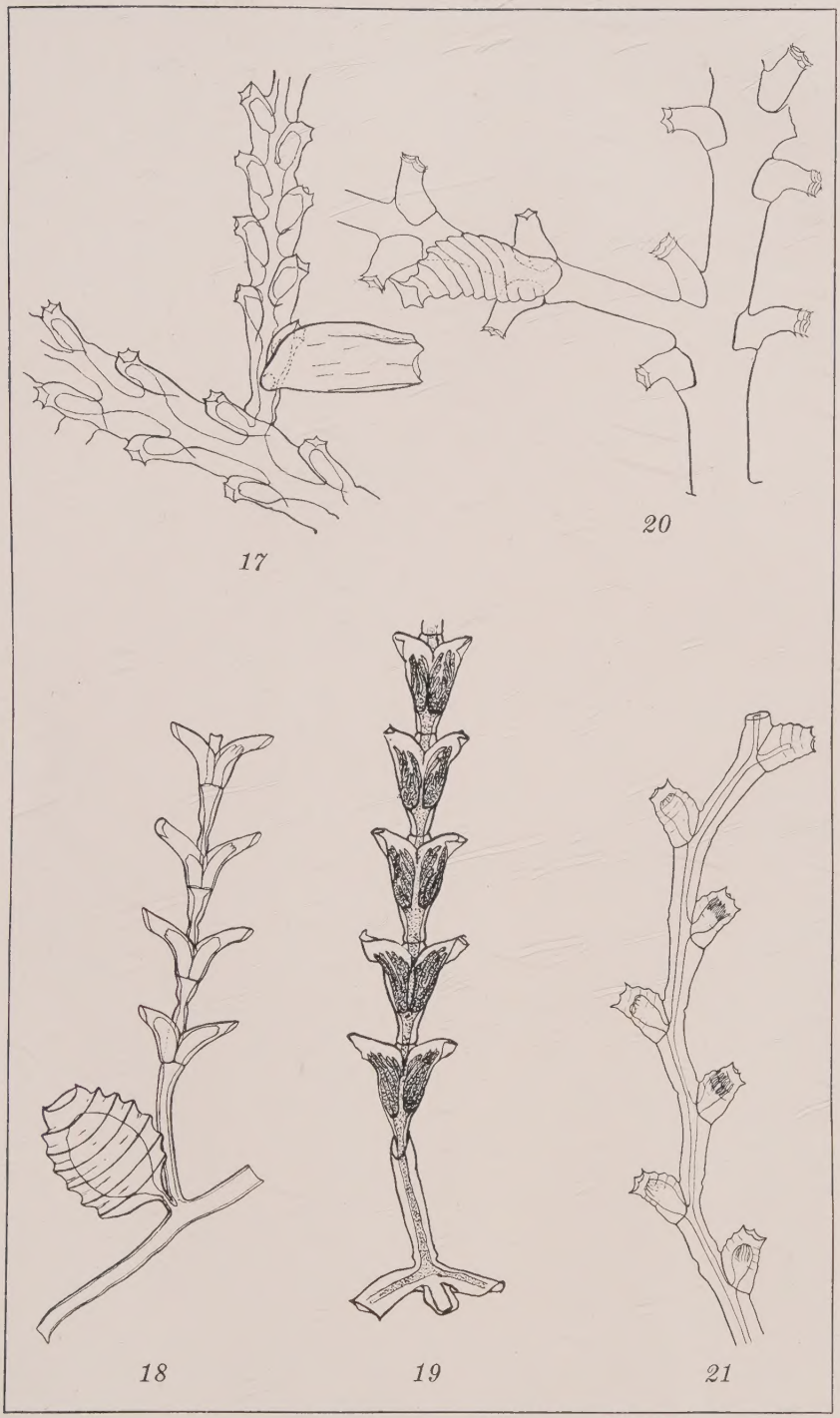


PLATE 5.


PLATE 6.